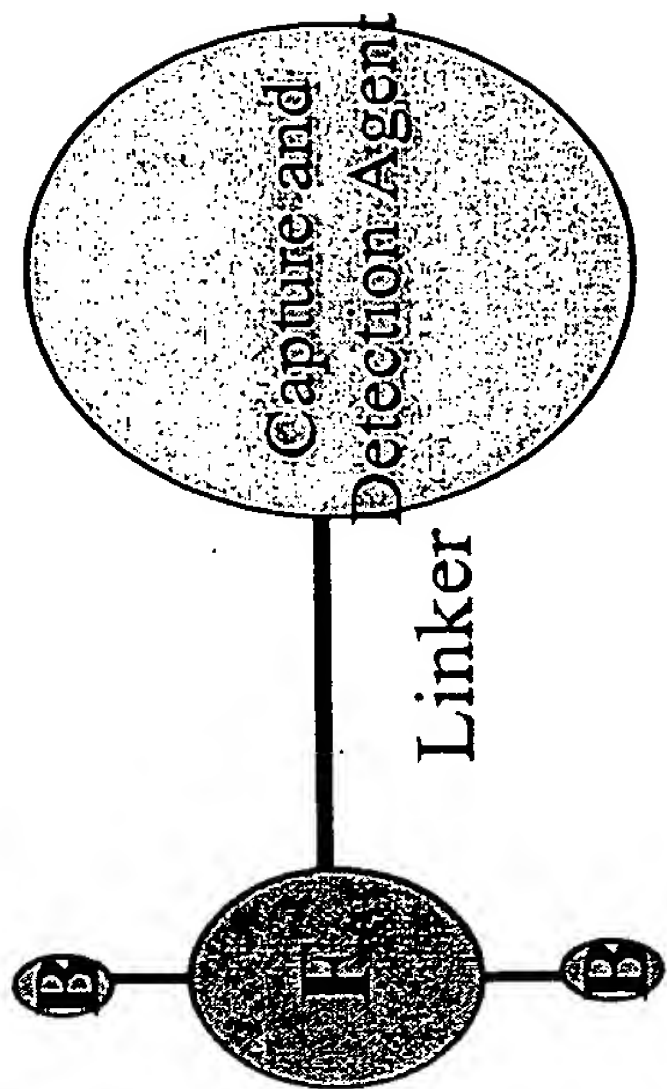
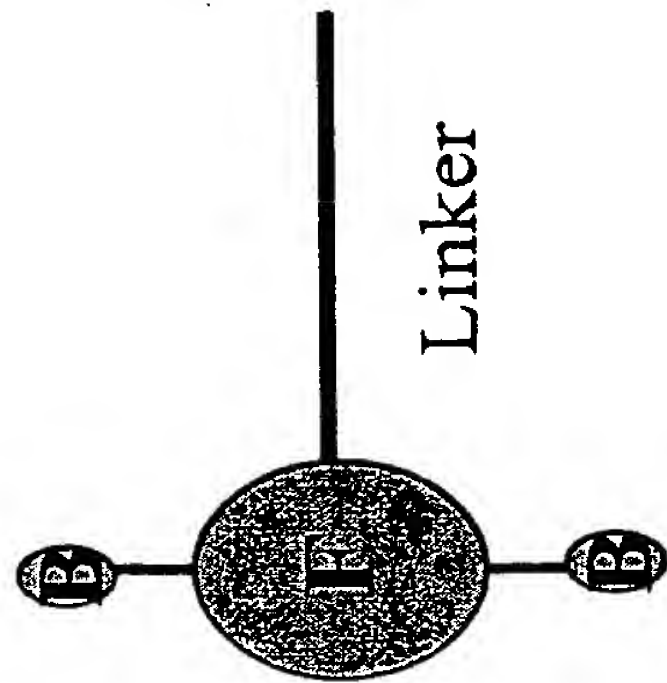




RECEIVED
APR 17 2003
TECH CENTER 1600/2900

1/24

High-throughput Target ID



Library of Bioactive
Compounds

Library of Target ID
Compounds

Use corresponding
activity-based probe to
identify the biological target

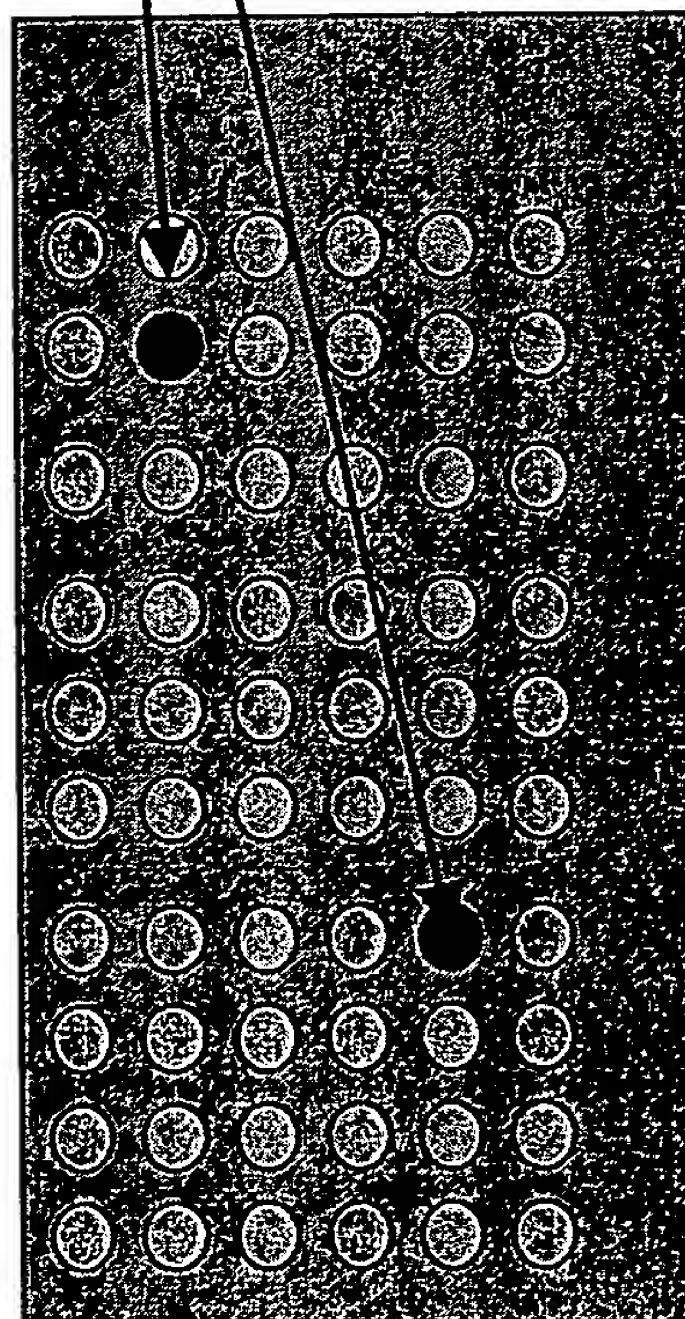


FIGURE 1



2/24

PyS Δ PyS MeS MeS Δ

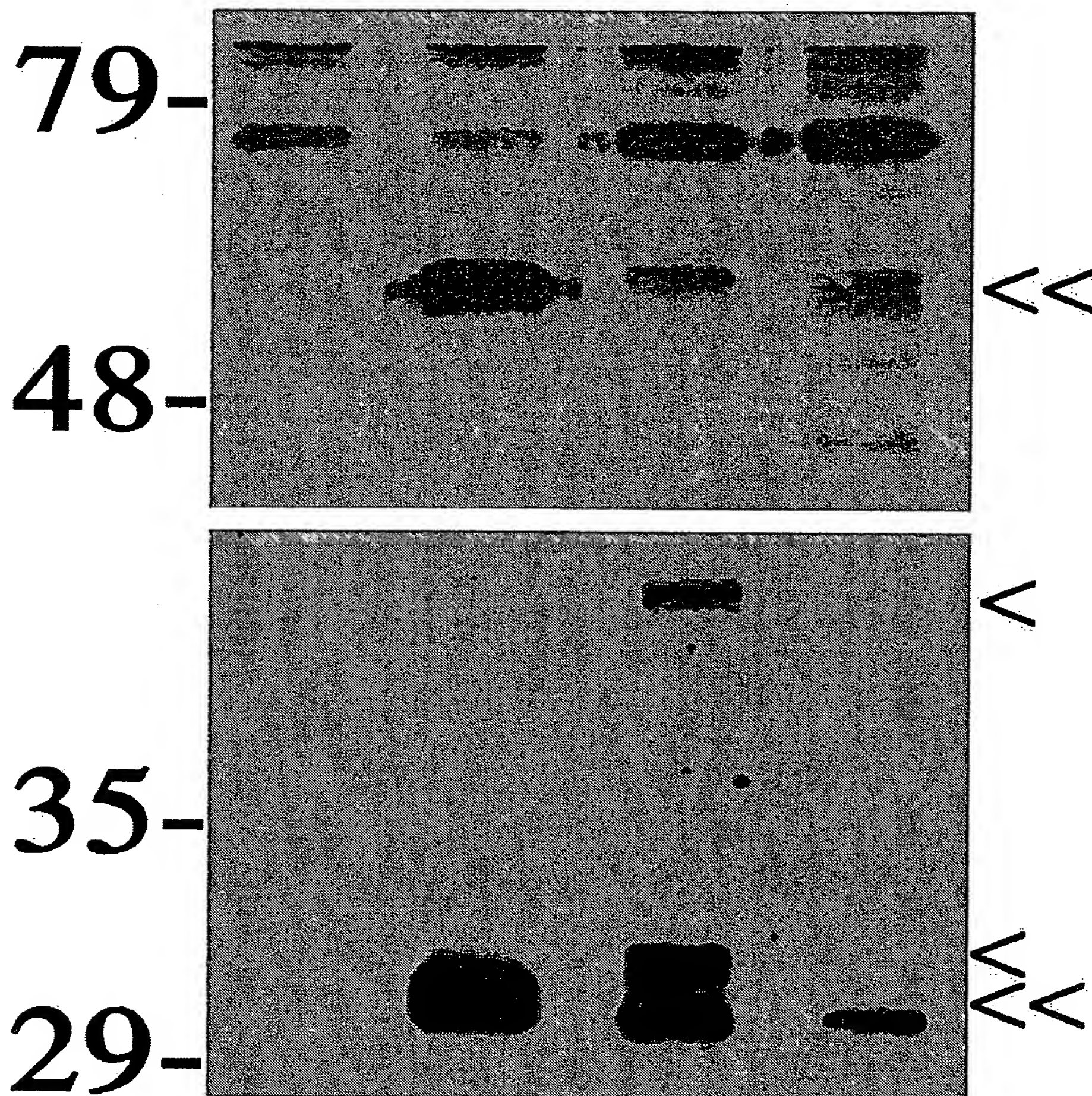


FIGURE 2



3/24

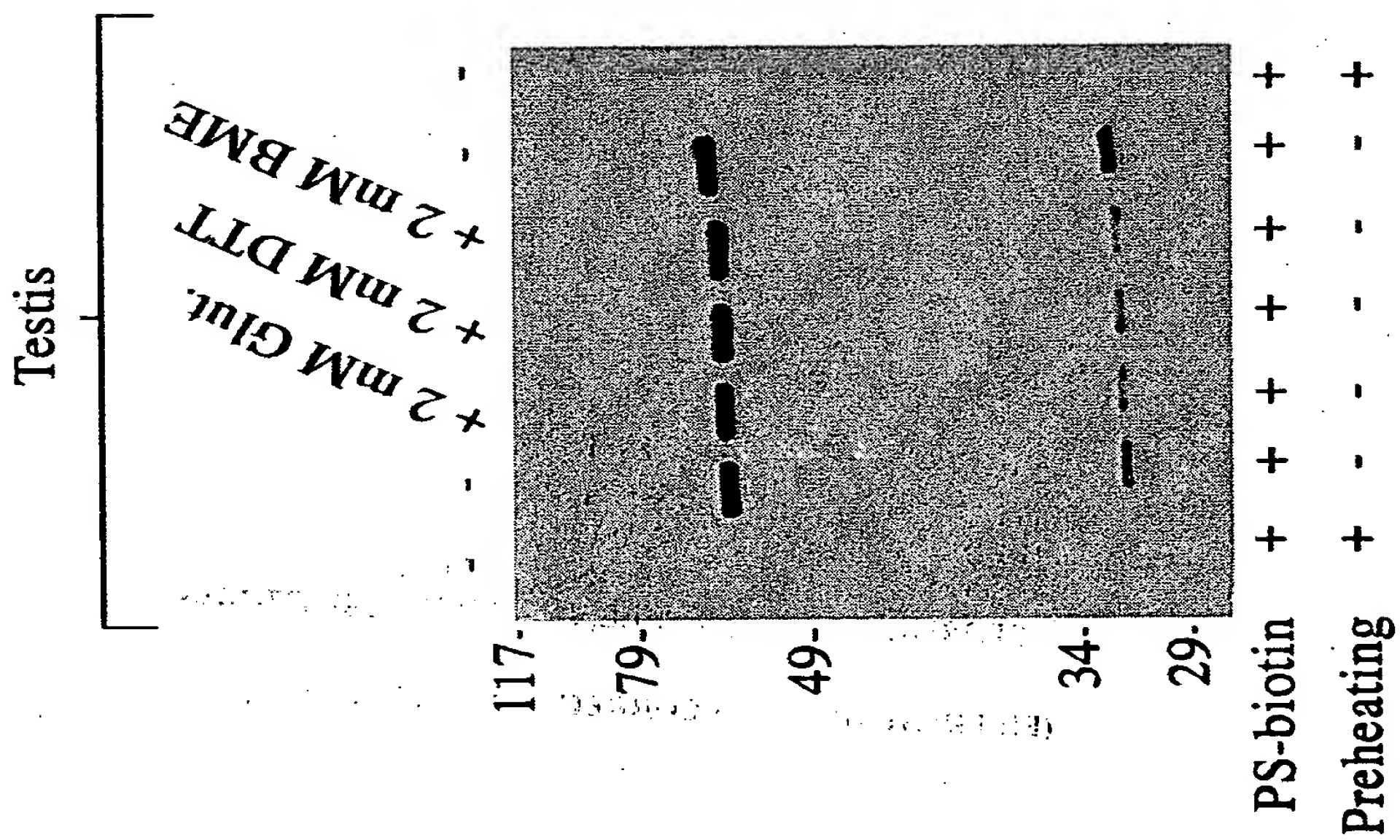
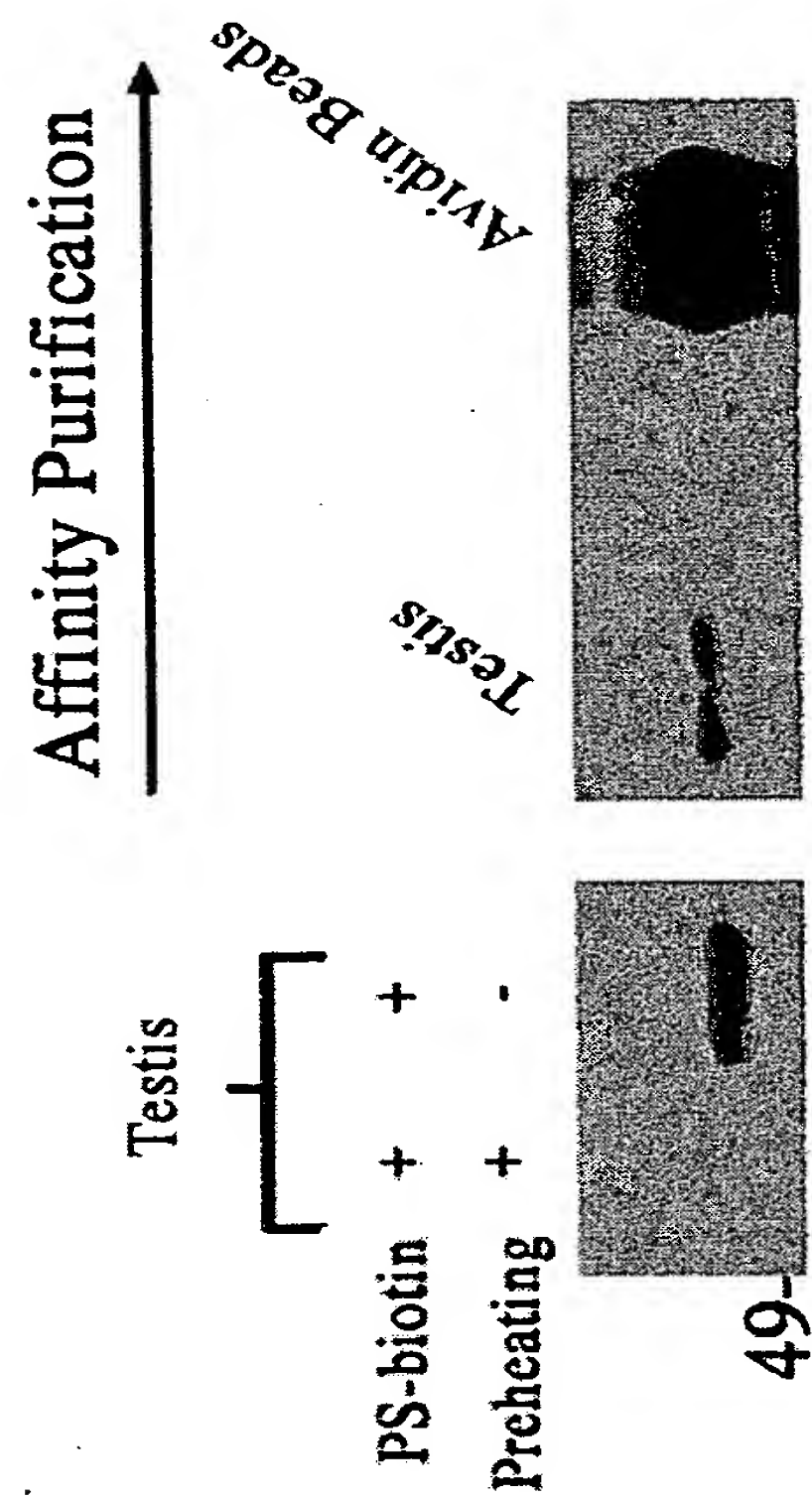
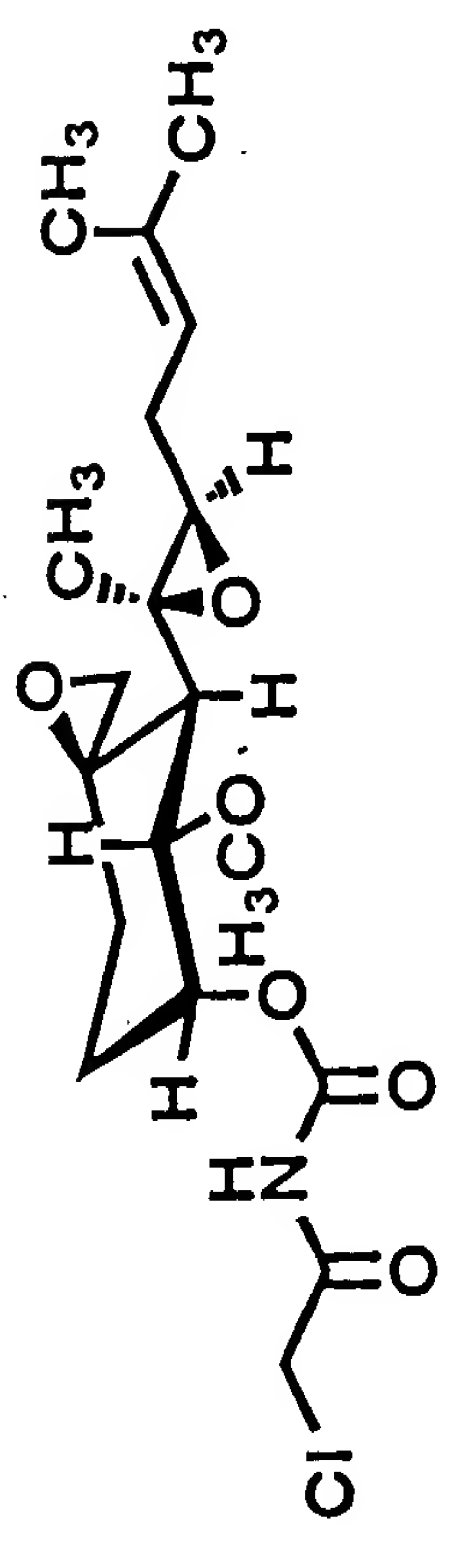


FIGURE 3

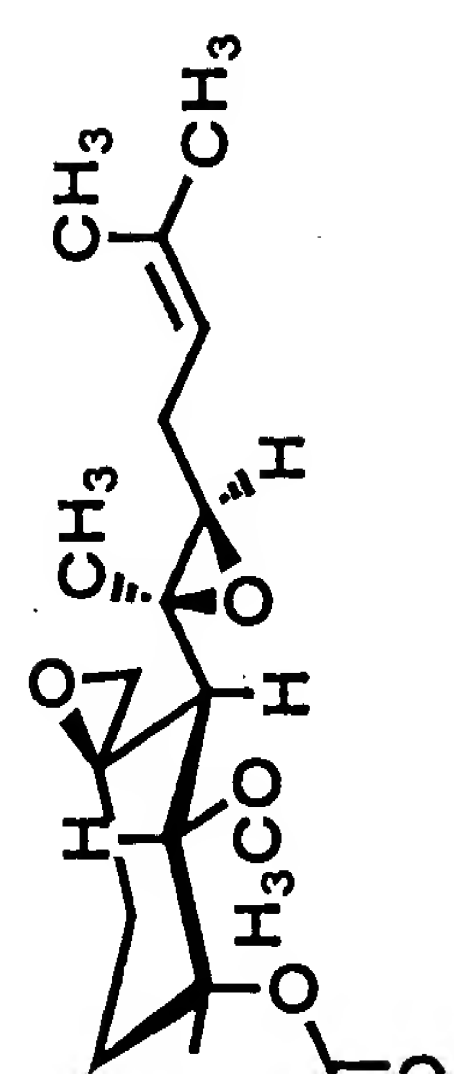


4/24

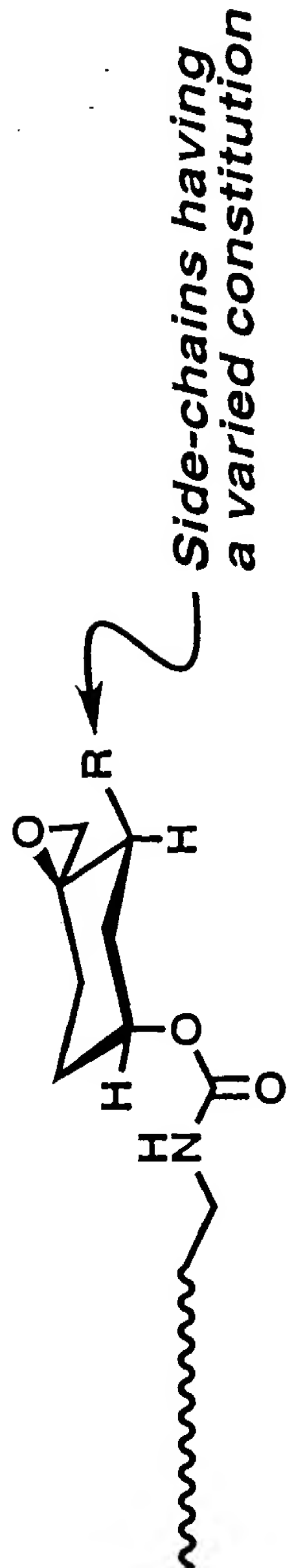
RECEIVED
APR 17 2003
TECH CENTER 1600/2900



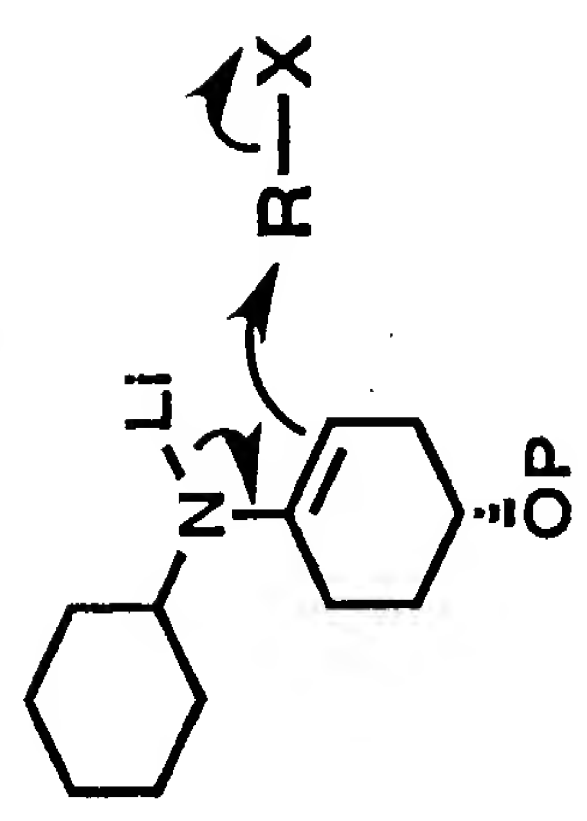
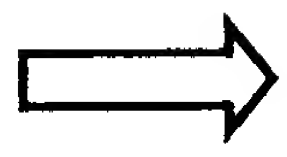
TNP-470



magillin

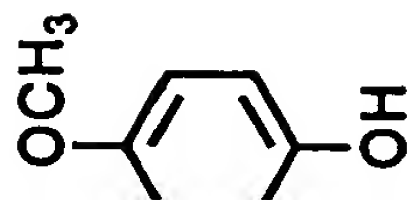
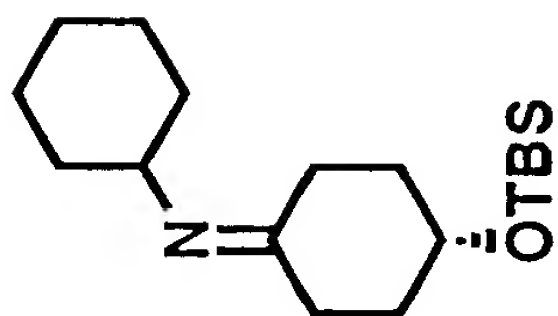


Side-chains having a varied constitution



X = halide
R = various aliphatic, alicyclic, alkyl aryl, or alkyl heteroaryl groups
P = unspecified protecting group

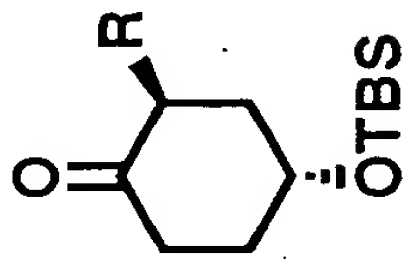
FIGURE 4A



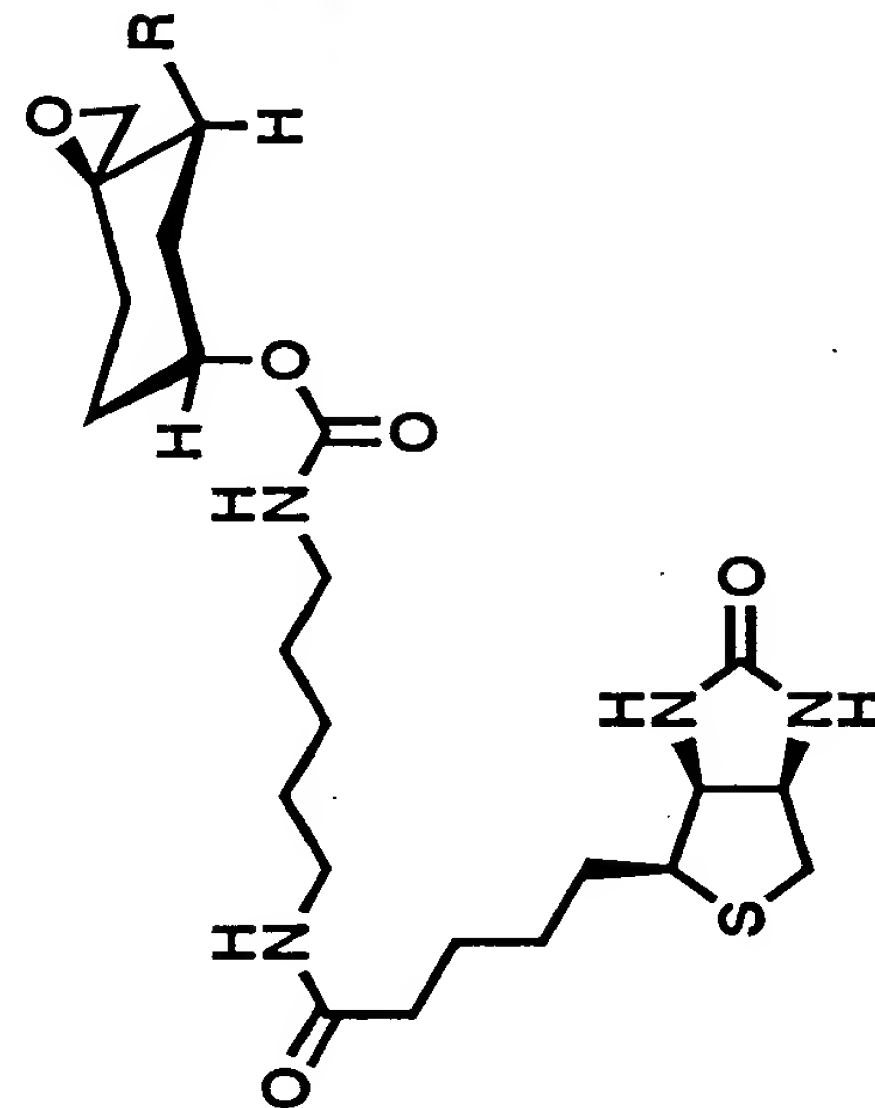
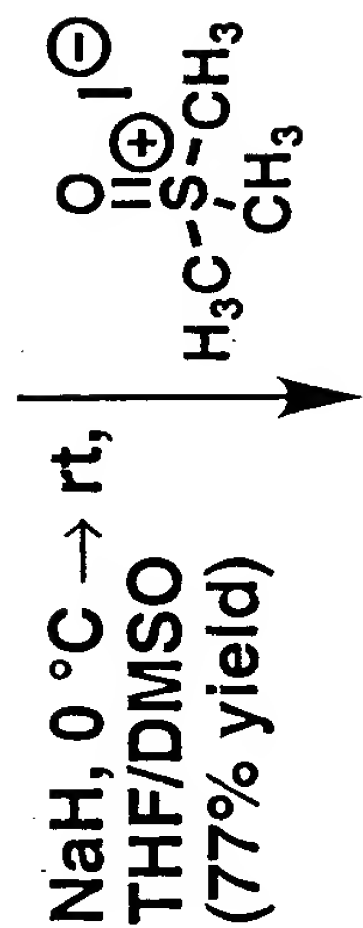
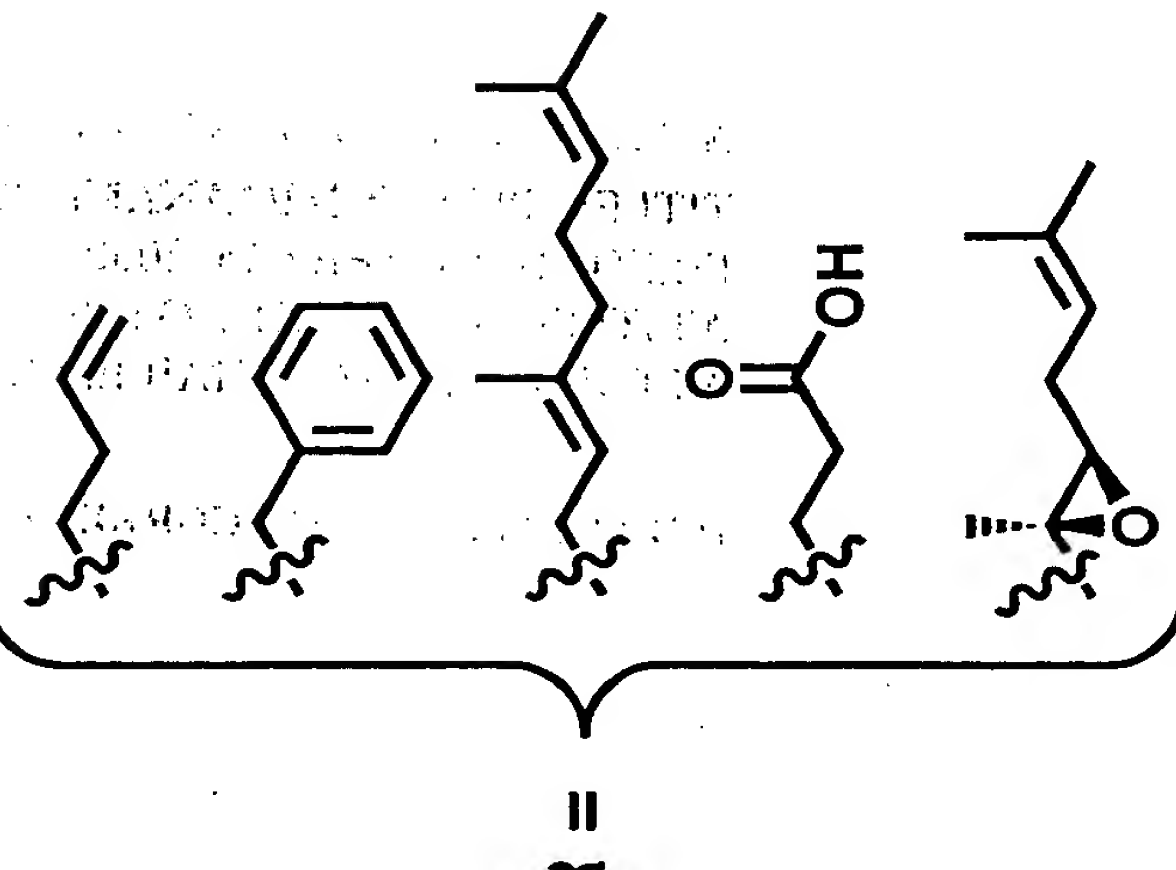
4 steps
(ca. 70% yield)

LDA, THF, 0 °C;

then R-Br, 0 °C → rt
(75% yield)



[major component of
a 2:1 mixture of epimers]



1. *n*-Bu₄NF,
THF (95%)

2. NHS carbonate,
Et₃N, CH₃CN
3. Biotin-NH₂,
MeOH (72%)

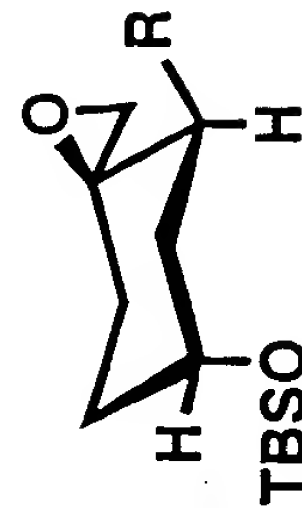


FIGURE 4B



6/24

RECEIVED
APR 17 2003
TECH CENTER 1600/2900

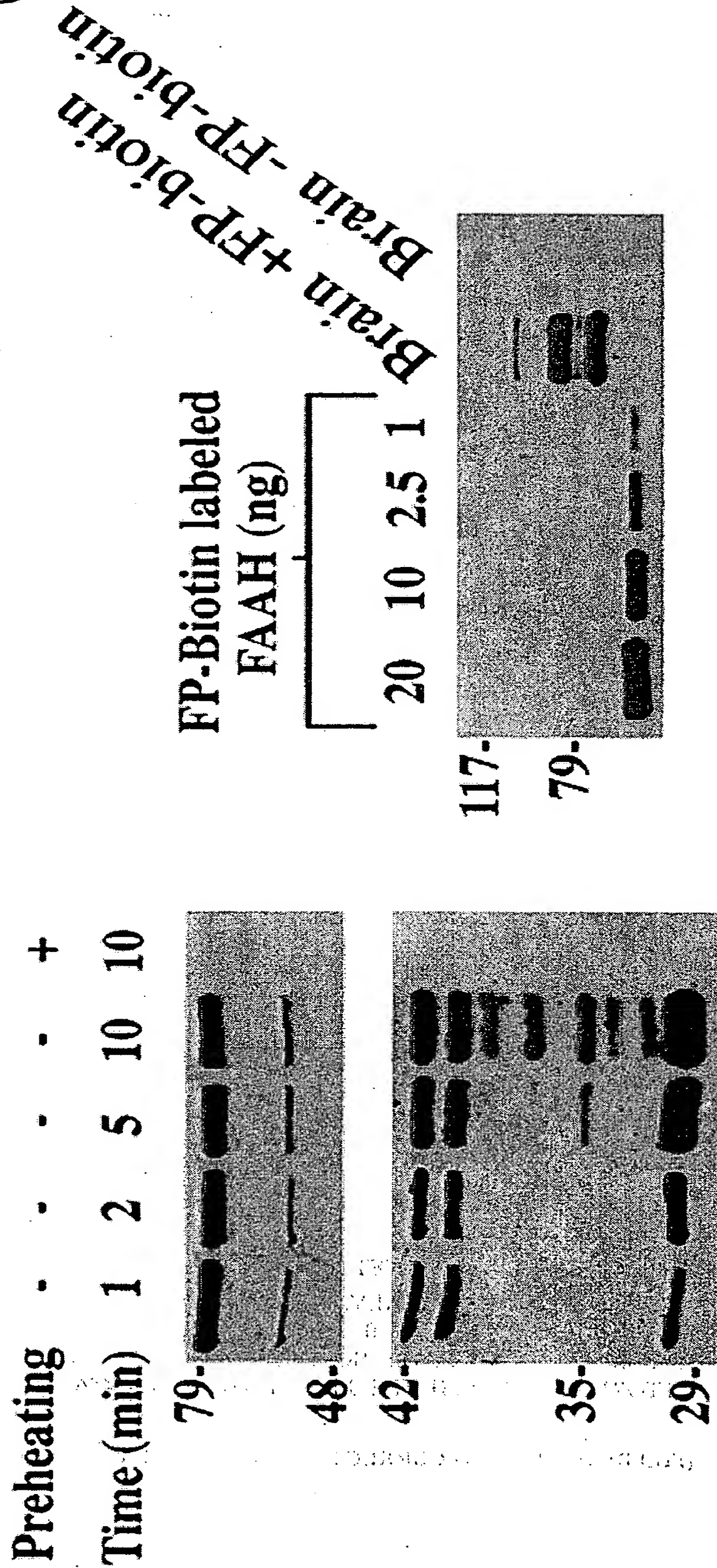


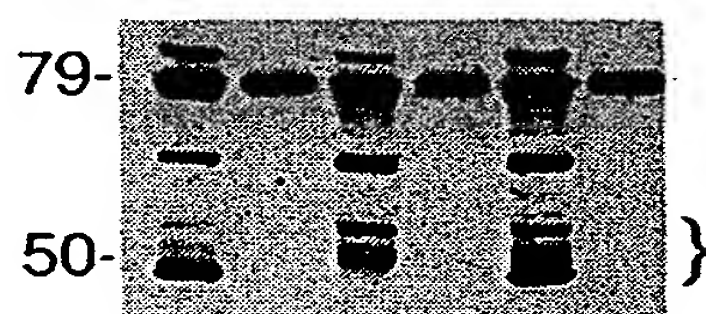
FIGURE 5



7/24

RECEIVED
APR 17 2003
TECH CENTER 1600/2900

FP-peg-biotin (μ M)	4	4	0	0	2	2
FP-biotin (μ M)	0	0	4	4	2	2
Preheated	-	+	-	+	-	+



FP-peg-biotin	-	-	+	+
FP-biotin	+	+	-	-
Preheated	+	-	-	+

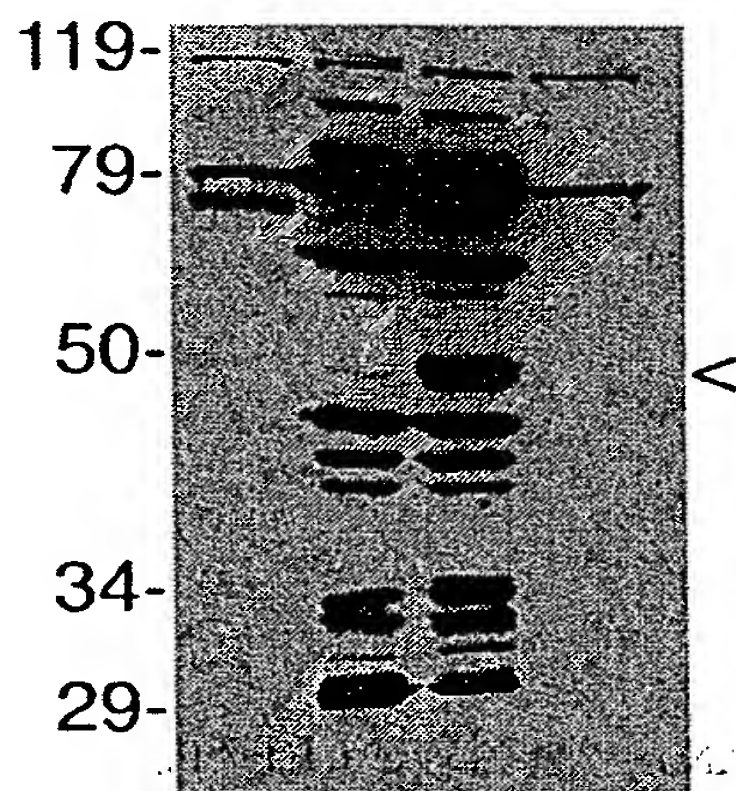


FIGURE 6



8/24

RECEIVED
APR 17 2003
TECH CENTER 1600/2900

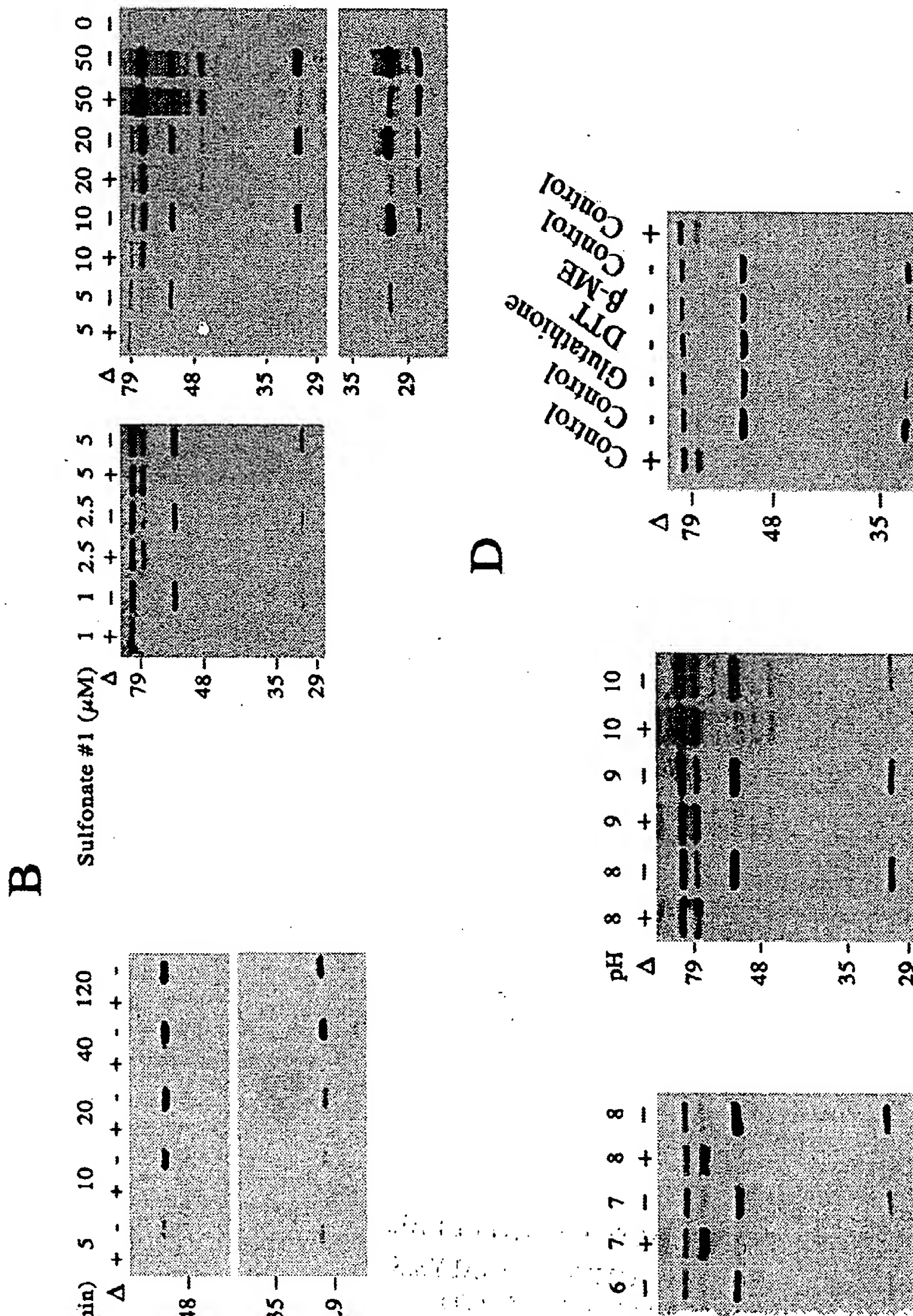
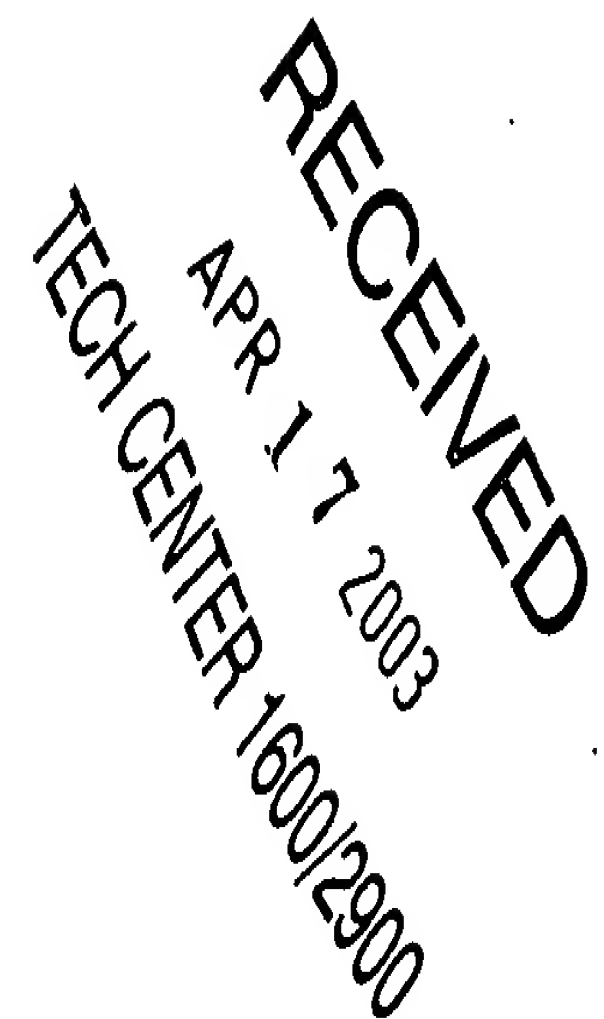


FIGURE 7





10/24

RECEIVED
APR 17 2003
TECH CENTER 1600/2900

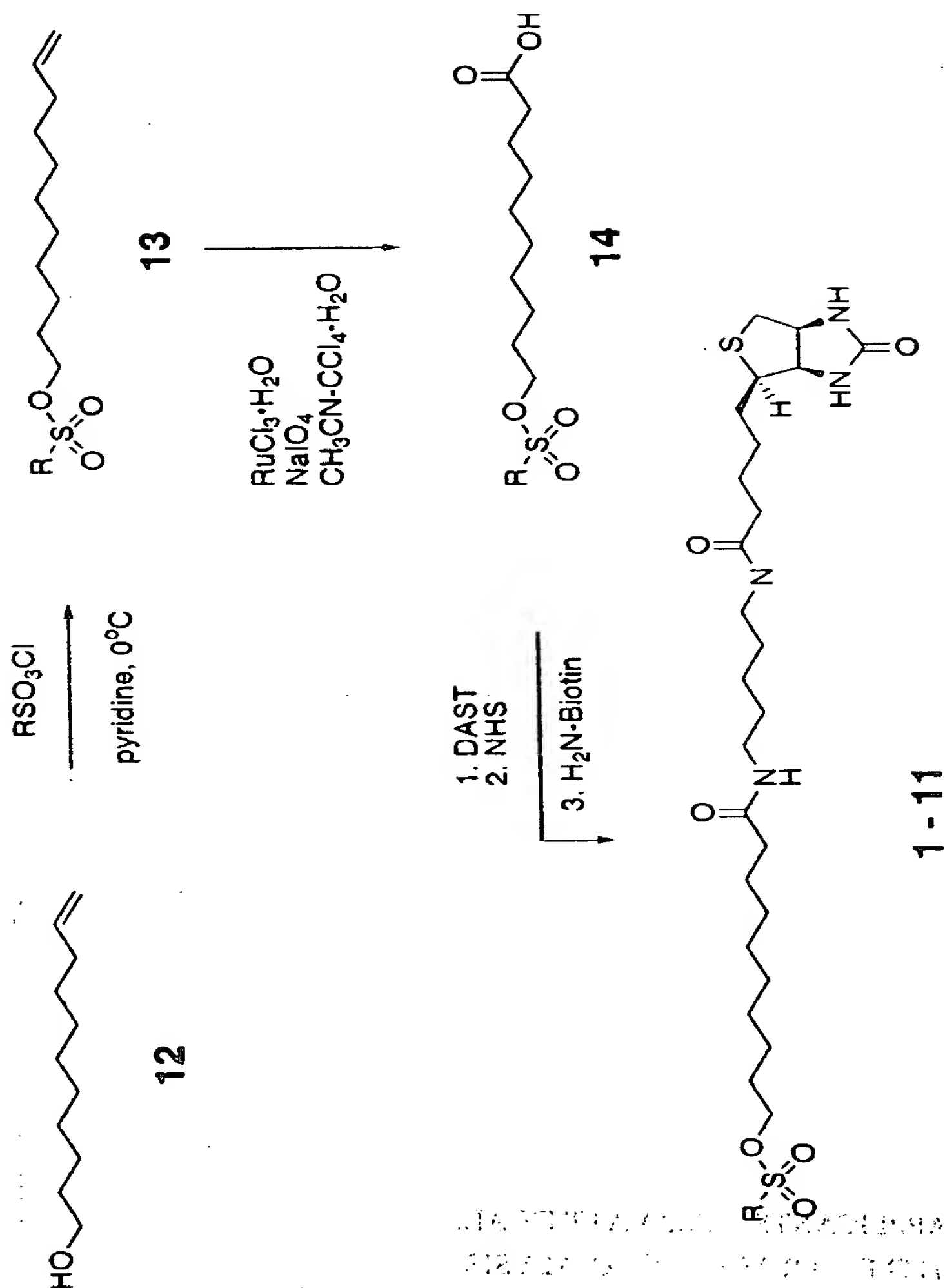


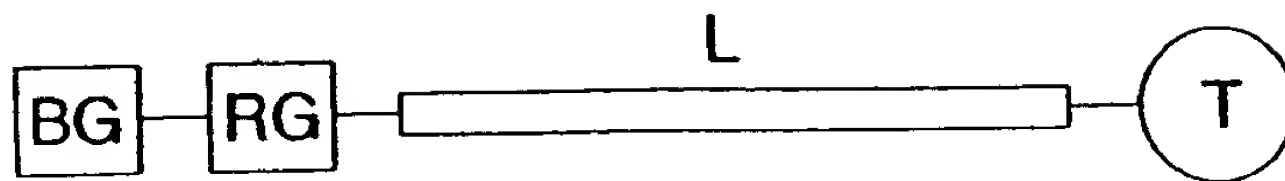
FIGURE 9



11/24

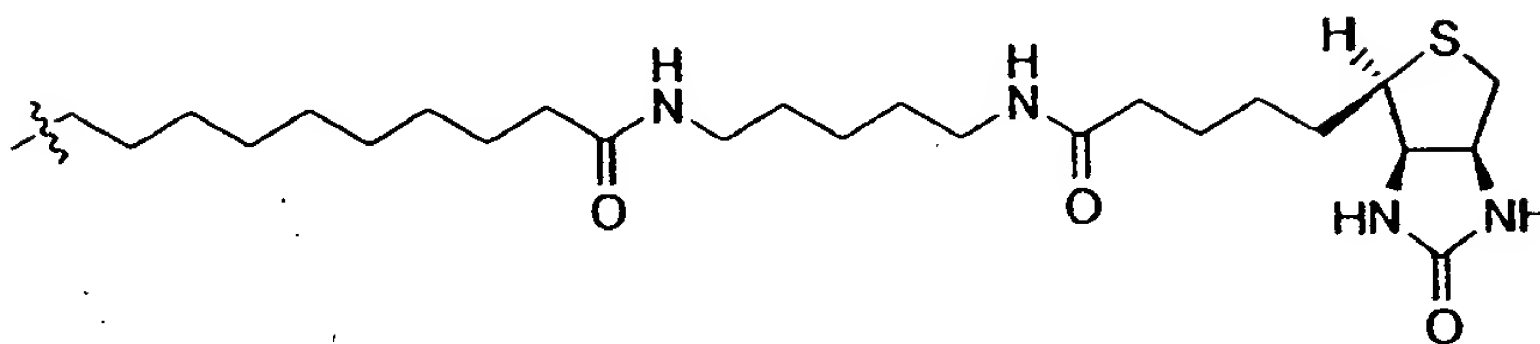
RECEIVED
APR 17 2003
TECH CENTER 1600/2900

A.

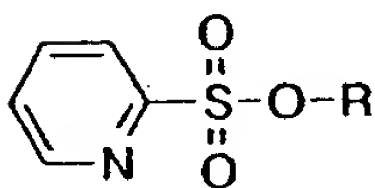


B.

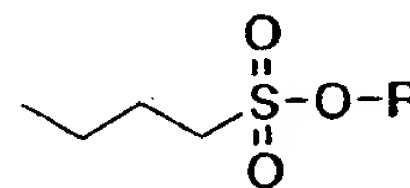
R =



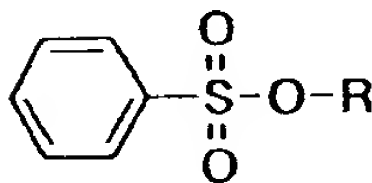
1



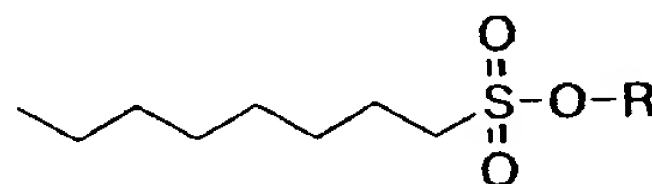
6



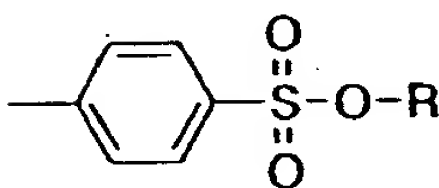
2



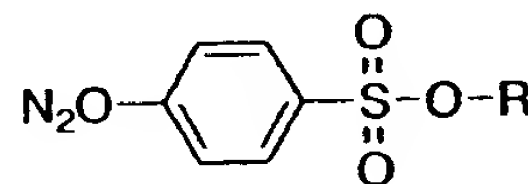
7



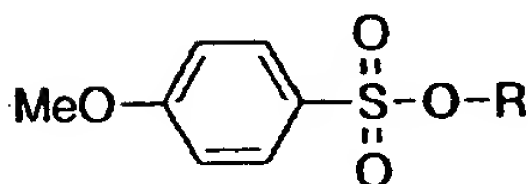
3



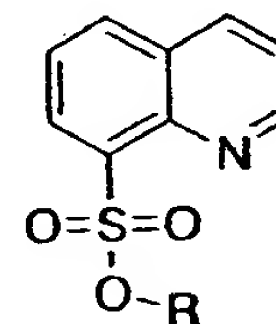
8



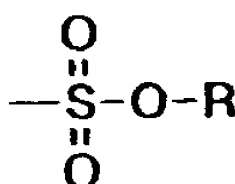
4



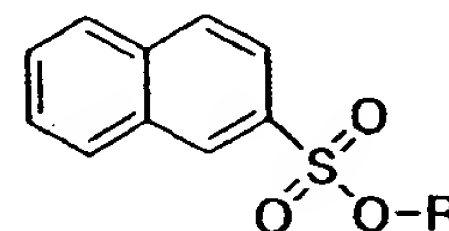
9



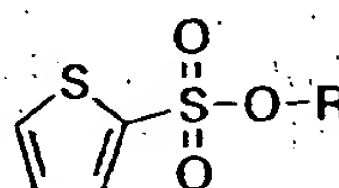
5



10



11

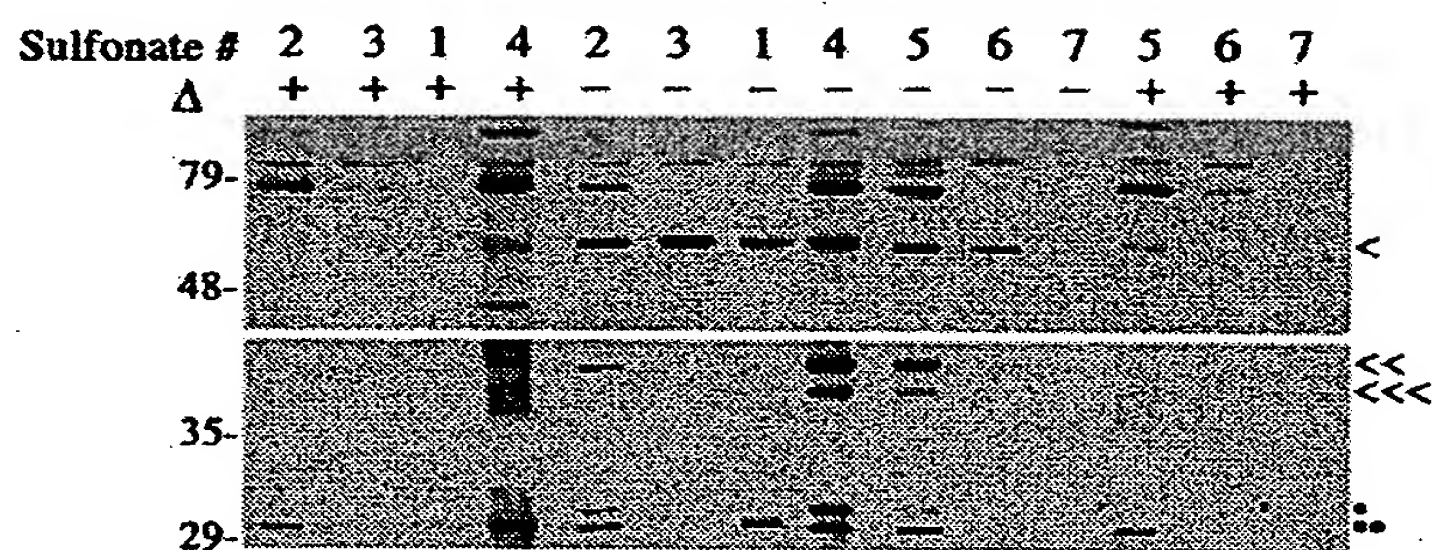




12/24

RECEIVED
APR 17 2003
TECH CENTER 1600/2900

A



B

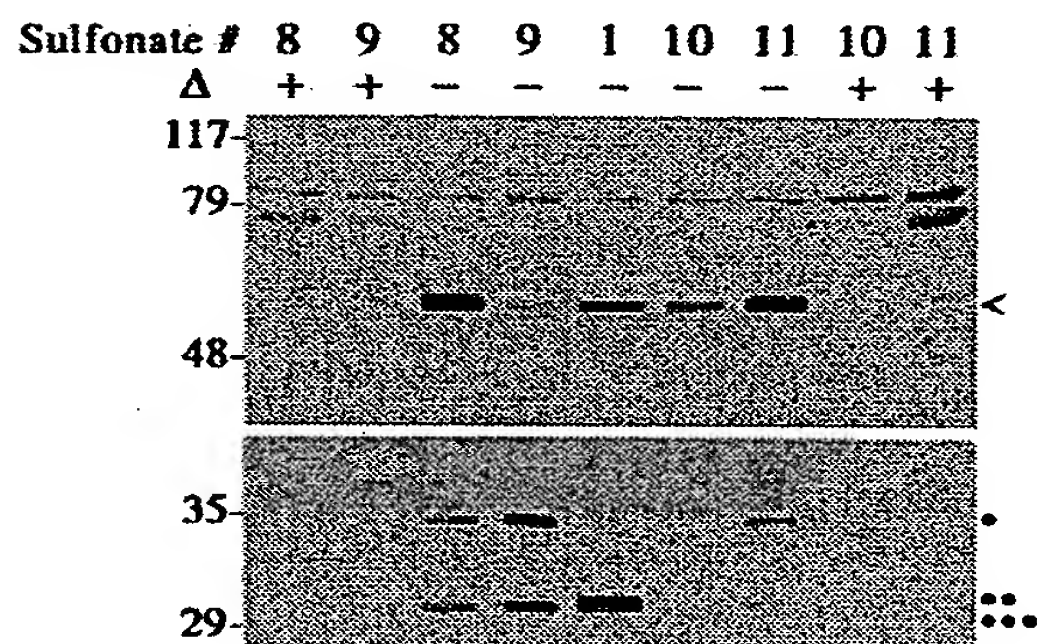


FIGURE 11

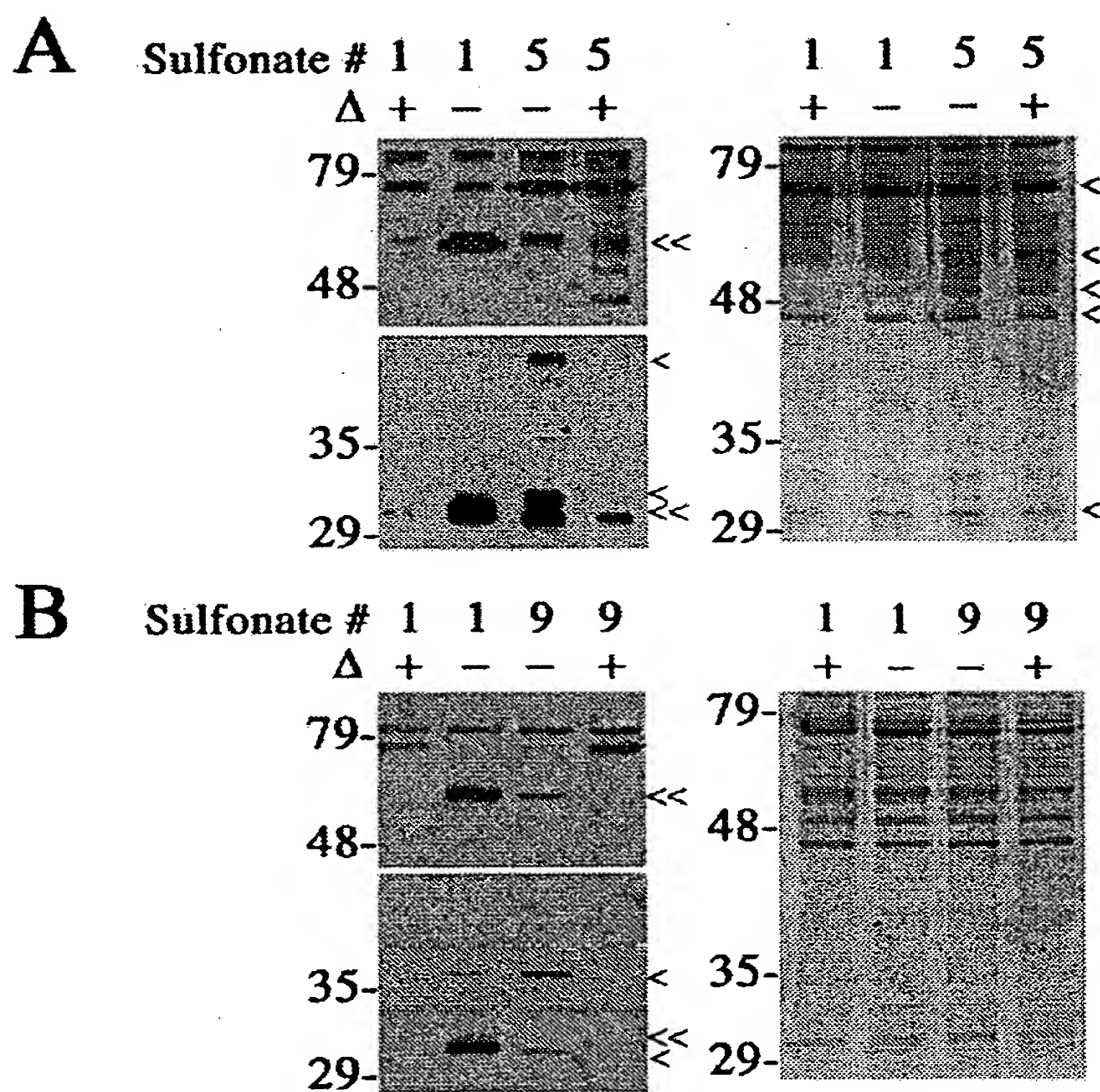
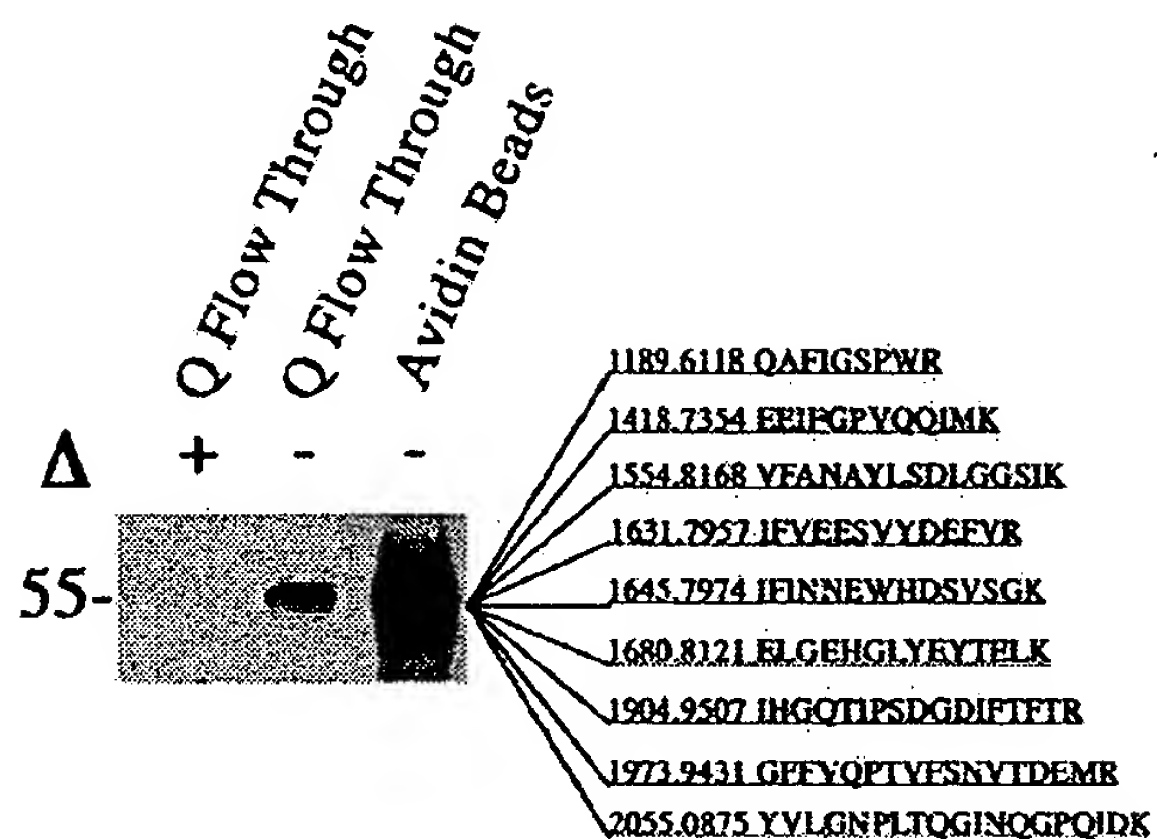


FIGURE 12

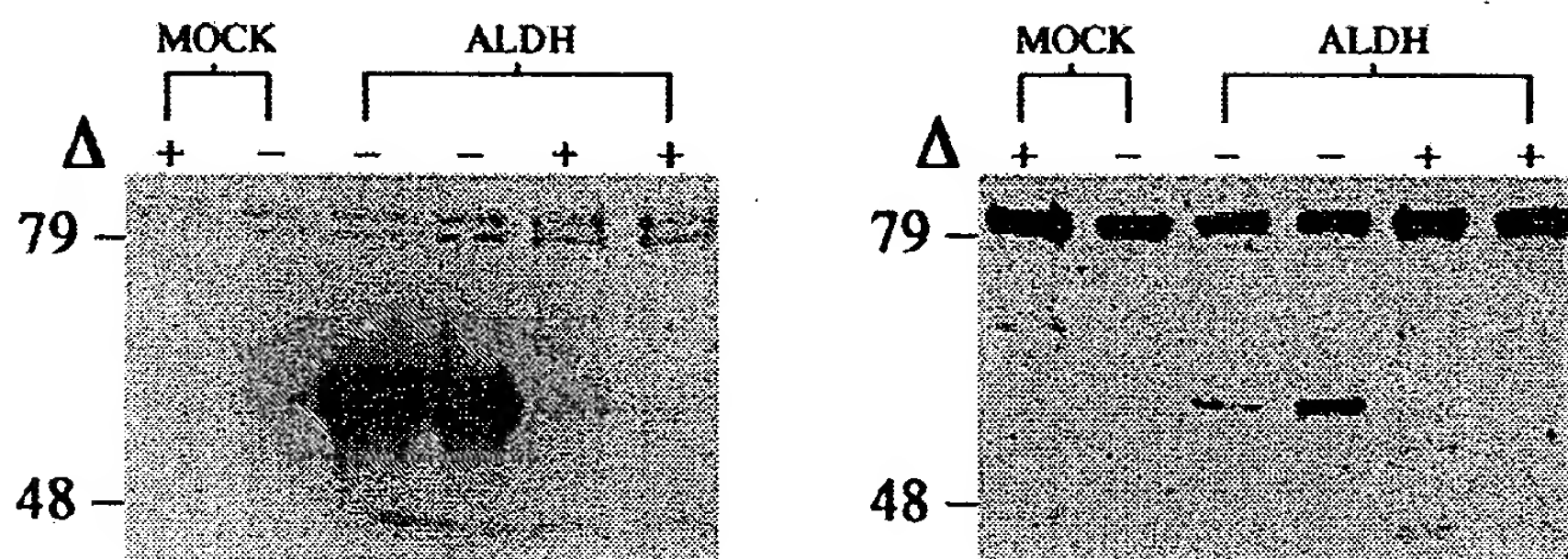


14/24

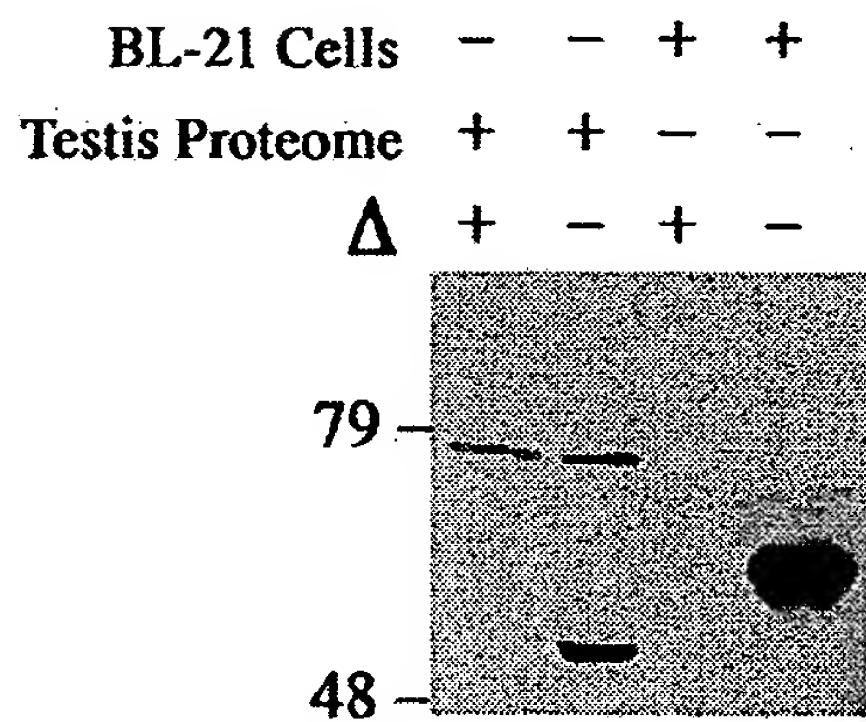
A



B



C



D

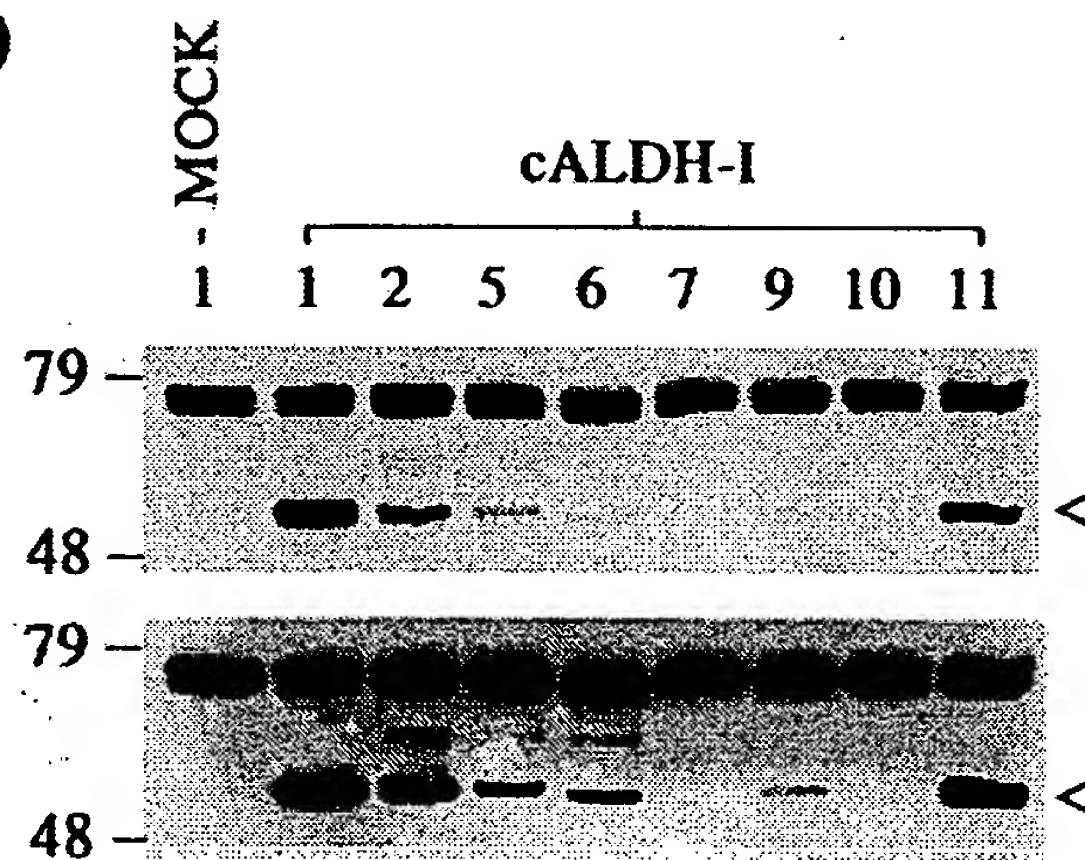
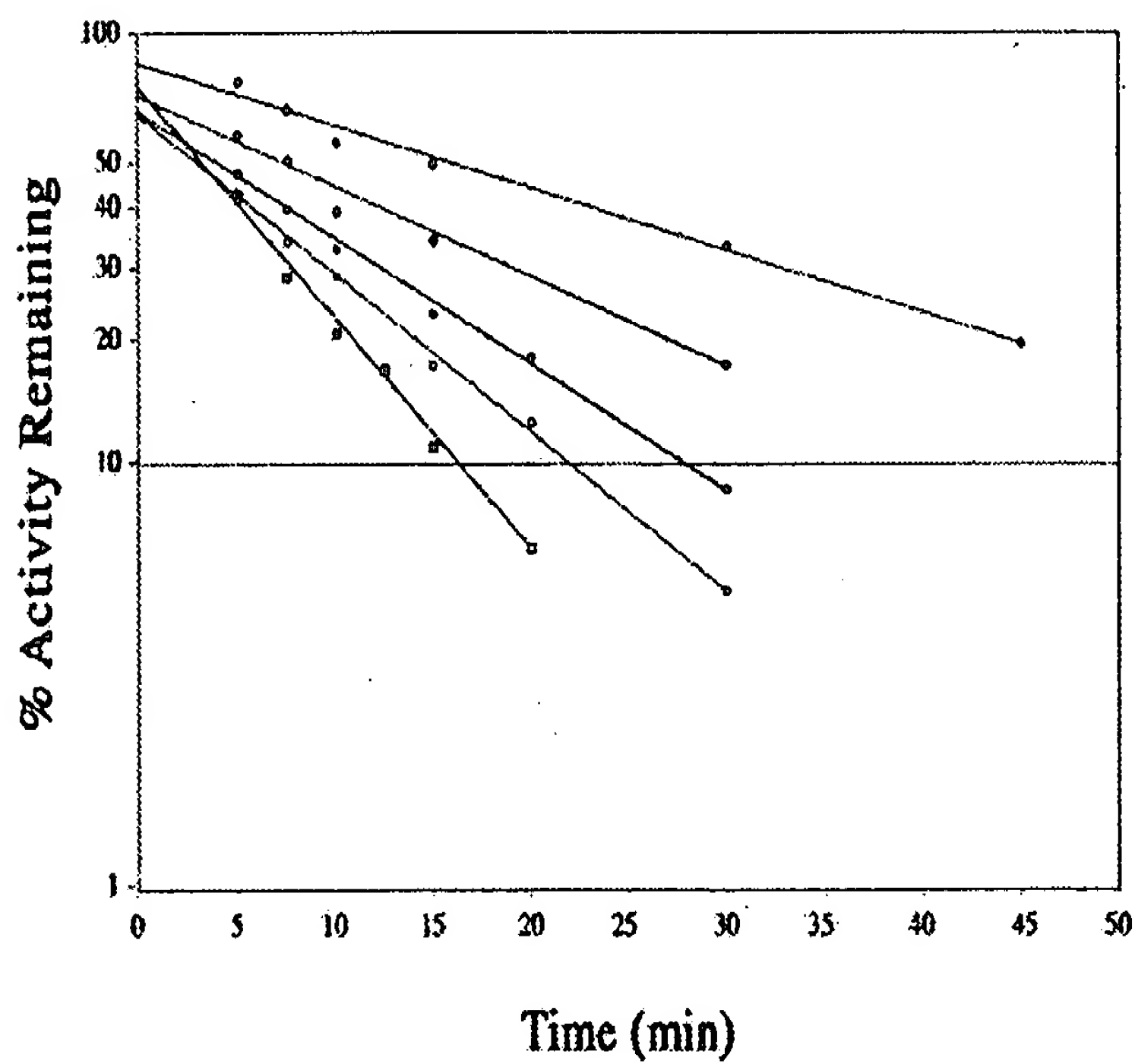


FIGURE 13



15/24

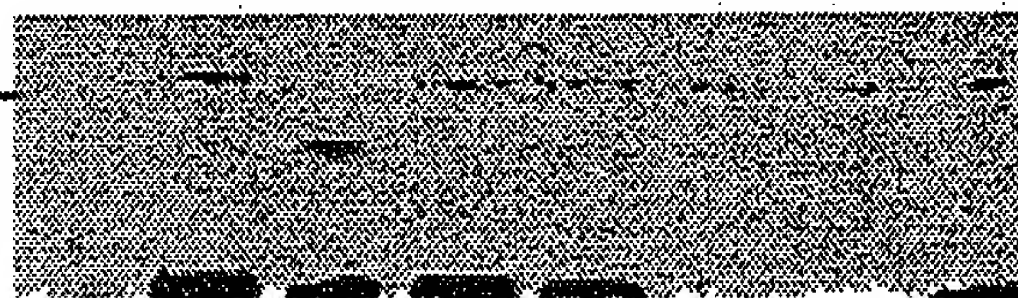
A



B

Competitor #	-	-	15	17	16	15	17	16
[Competitor (μ M)]	0	0	5	5	5	50	50	50
Δ	+	-	-	-	-	-	-	-

79 -





16/24

FP-biotin	-	-	+	+	+	+
Sulfonate #1	+	+	+	-	+	-
Δ	+	-	-	-	+	+

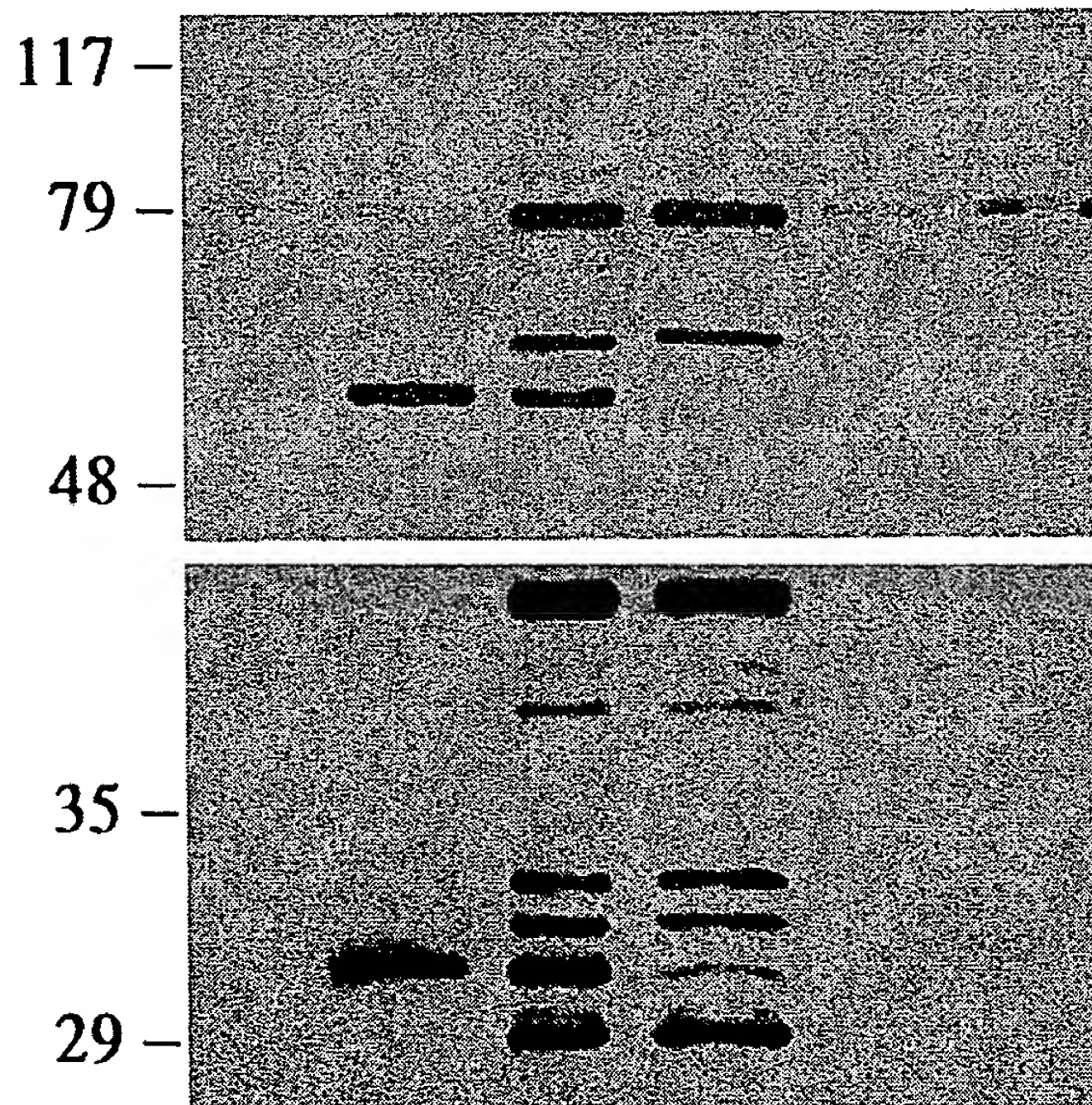
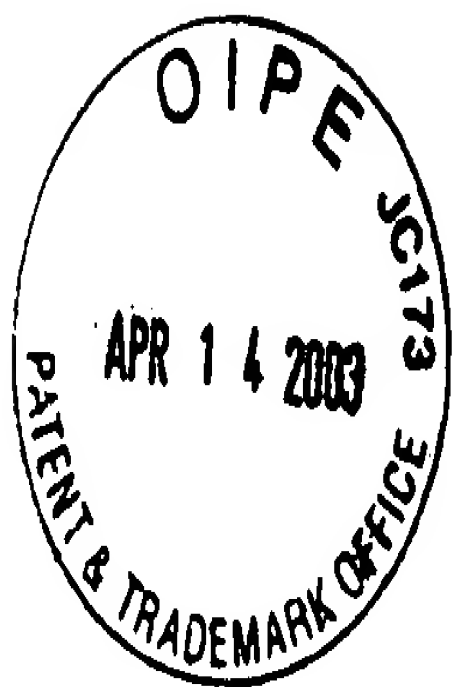
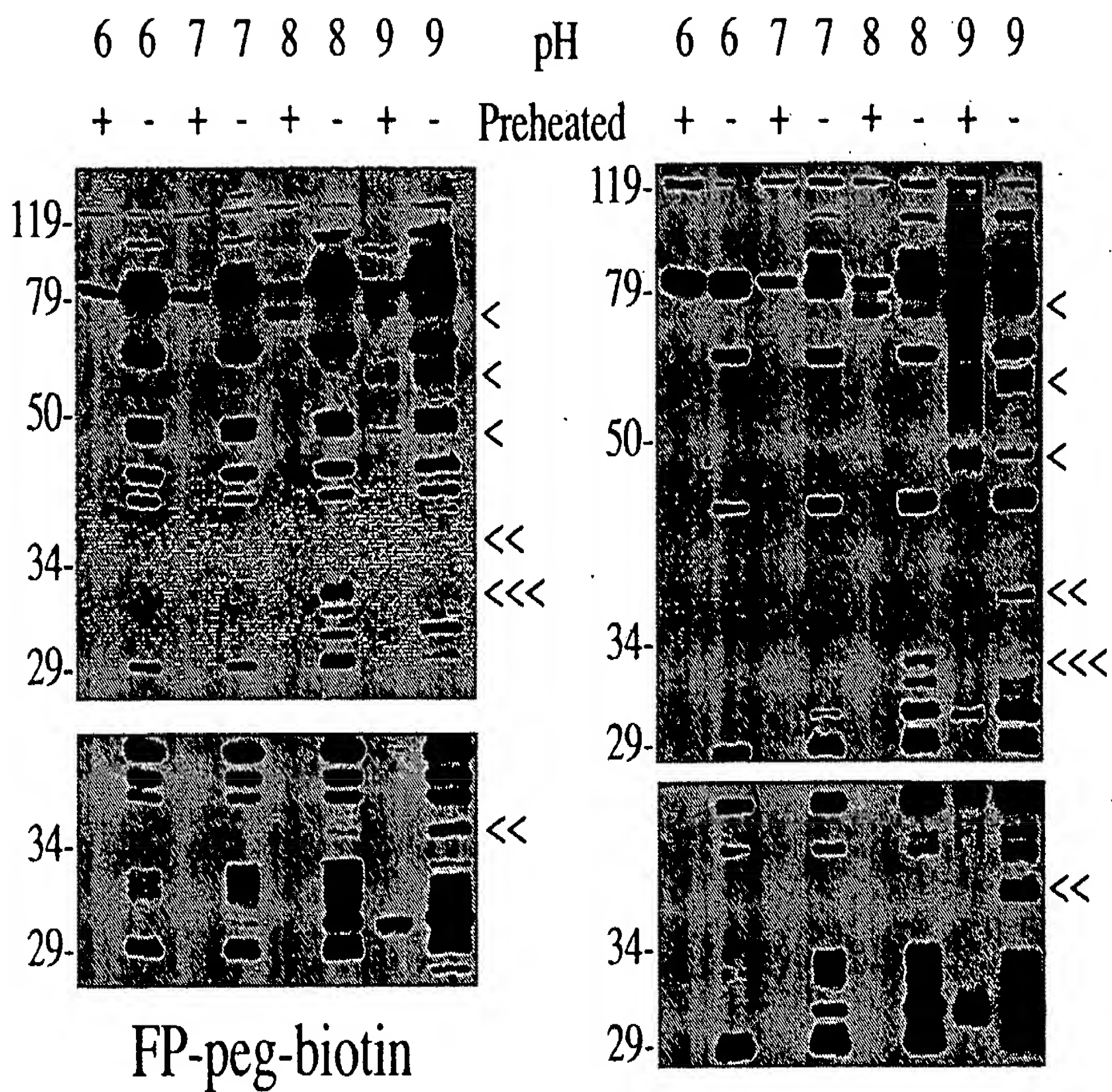


FIGURE 15



17/24





18/24

RECEIVED
APR 17 2003
TECH CENTER 1600/2900

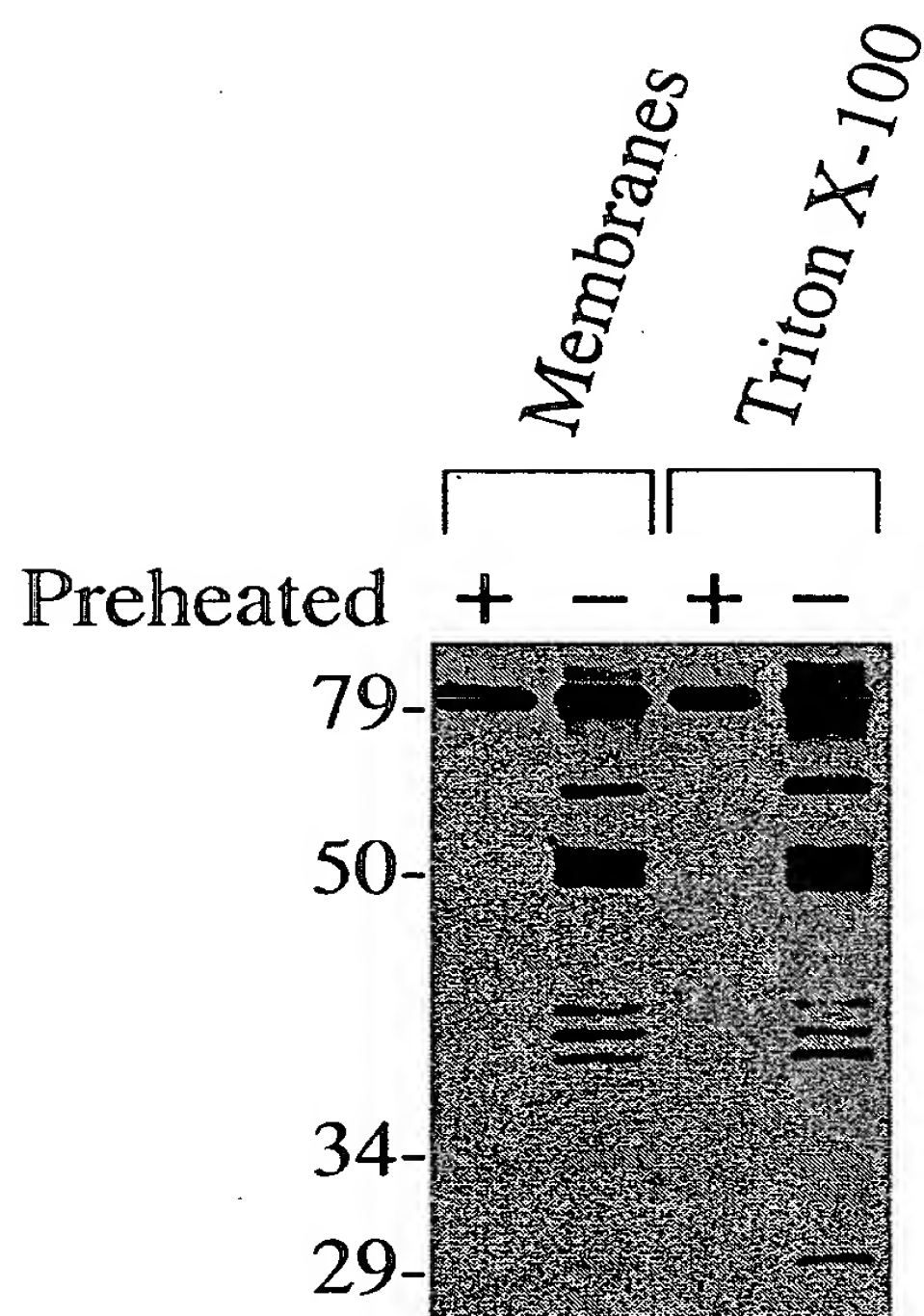


FIGURE 17

Western blot analysis of the effect of preheating on the binding of the anti-CD44 antibody to the cell surface. The cells were treated with the anti-CD44 antibody for 1 hour at 4°C or 37°C. The cells were then lysed and the lysate was immunoprecipitated with the anti-CD44 antibody. The immunoprecipitate was then analyzed by Western blotting with the anti-CD44 antibody. The results show that preheating the cells at 37°C for 1 hour significantly increases the binding of the anti-CD44 antibody to the cell surface.

Western blot analysis of the effect of preheating on the binding of the anti-CD44 antibody to the cell surface.



FP-peg-biotin	+	+	+	+	+	+	-	-	-
FP-biotin	-	-	-	-	-	-	+	+	+
OTFMK	0	0	200	50	5	1	0	200	50
Δ	+	-	-	-	-	-	-	-	-

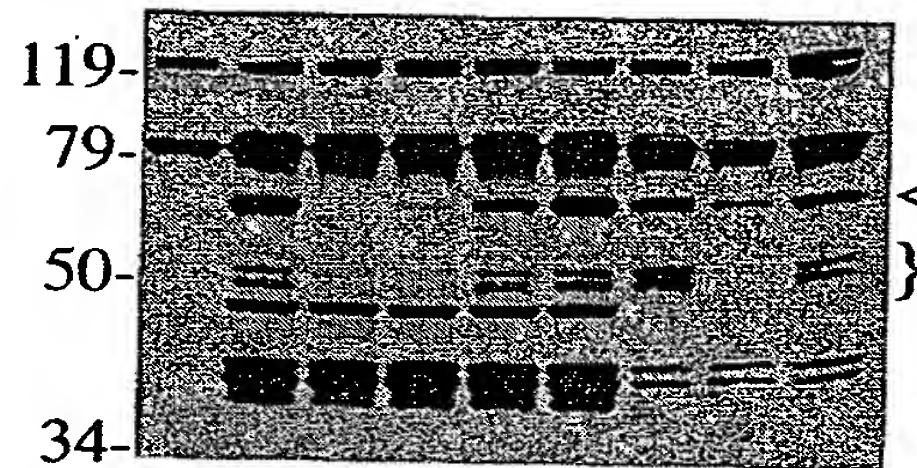


FIGURE 18



20/24

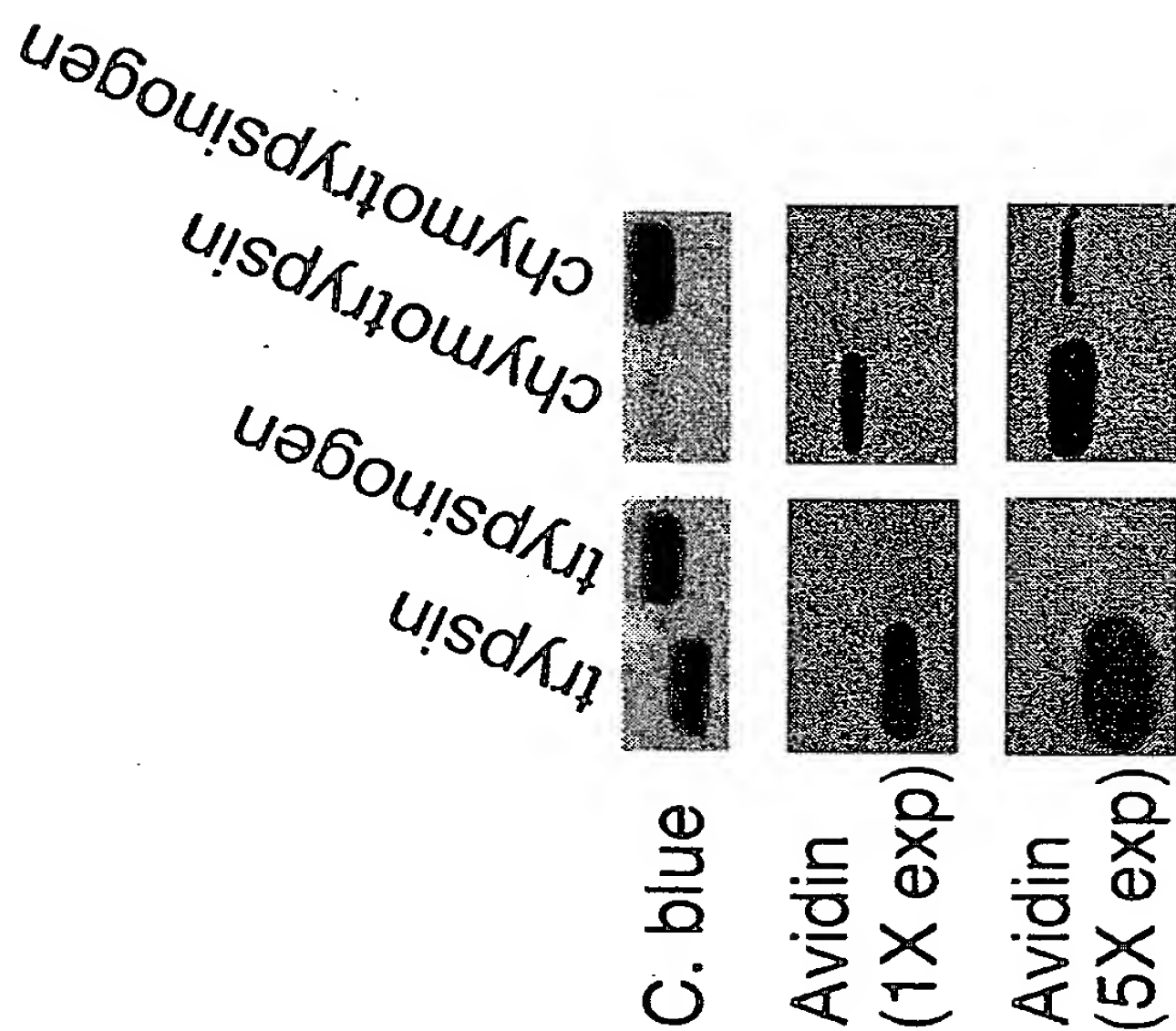


FIGURE 19

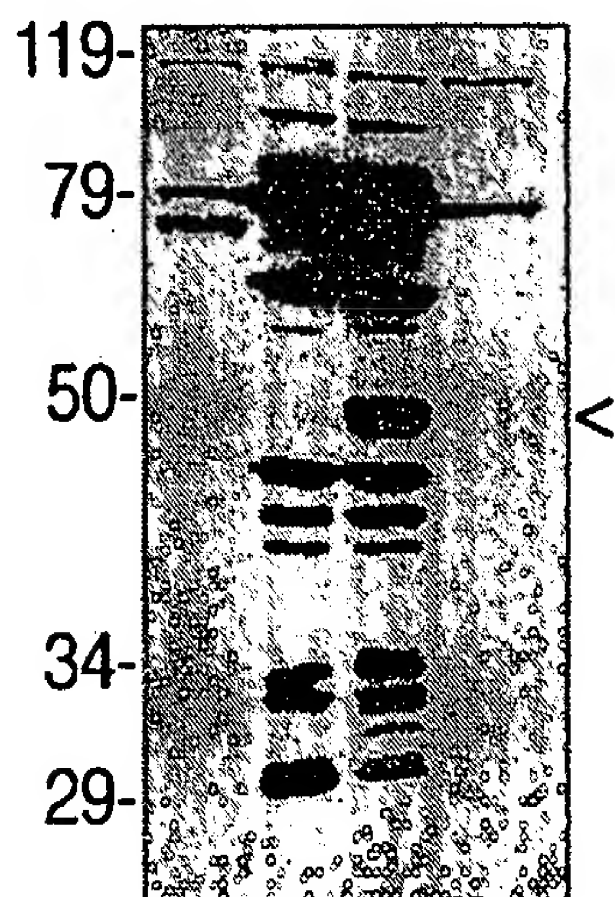


21/24

RECEIVED
APR 17 2003
TECH CENTER 1600/2900

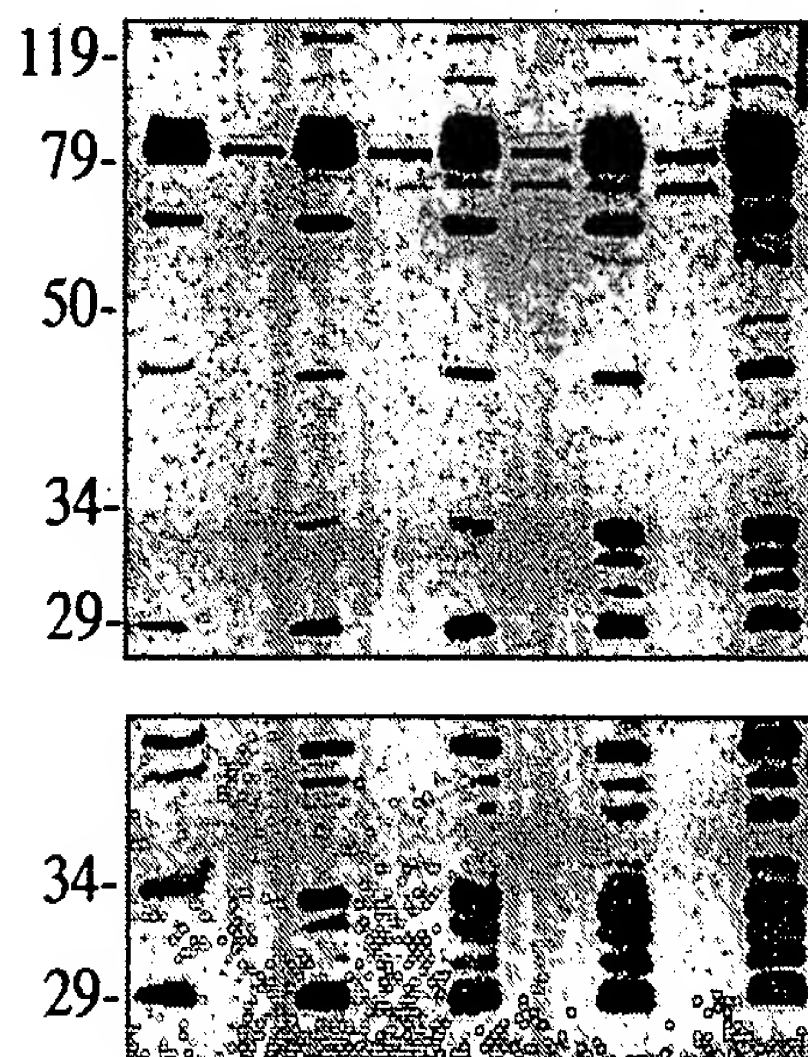
A.

FP-peg-biotin	-	-	+	+
FP-biotin	+	+	-	-
Preheated	+	-	-	+



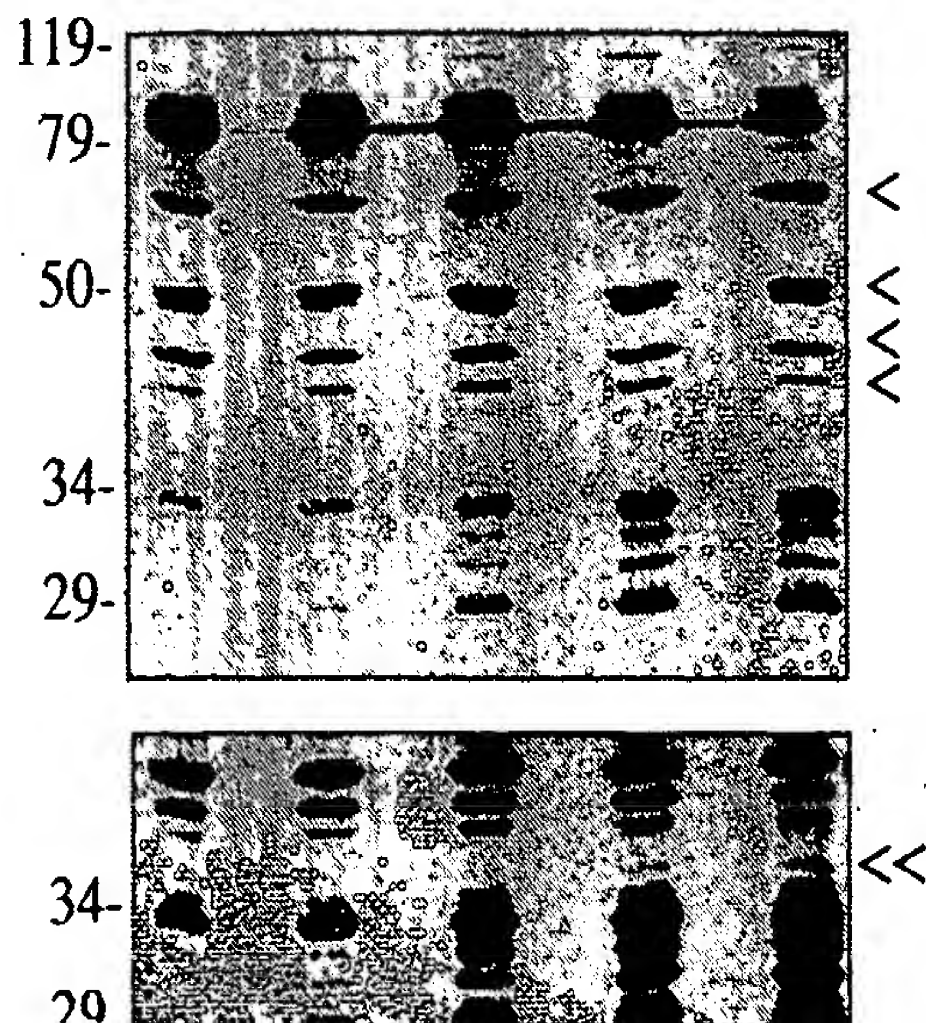
B.

FP-biotin (μ M)	0.5	1	1	2	2	4	4	8	8
Preheated	-	+	-	+	-	+	-	+	-



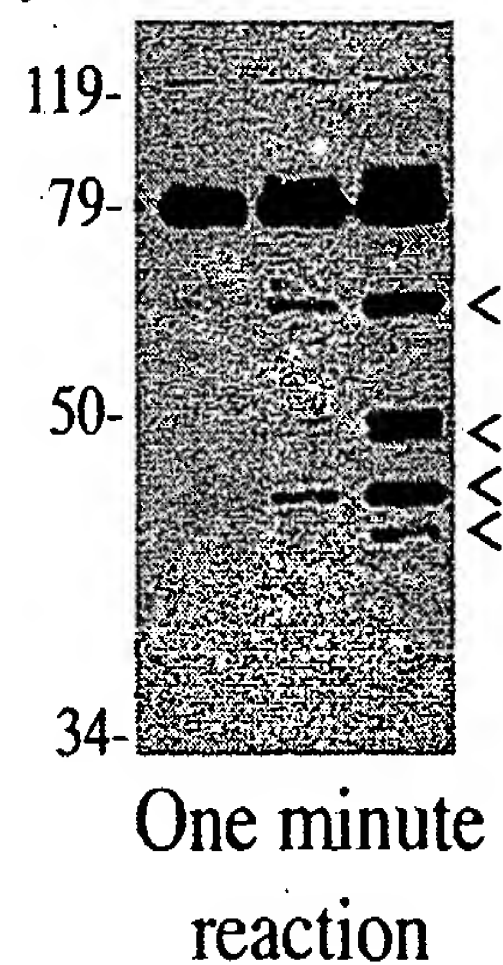
C.

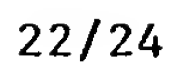
FP-peg-biotin (μ M)	0.5	1	1	2	2	4	4	8	8
Preheated	-	+	-	+	-	+	-	+	-



D.

FP-peg-biotin (μ M)	1	2	8
--------------------------	---	---	---



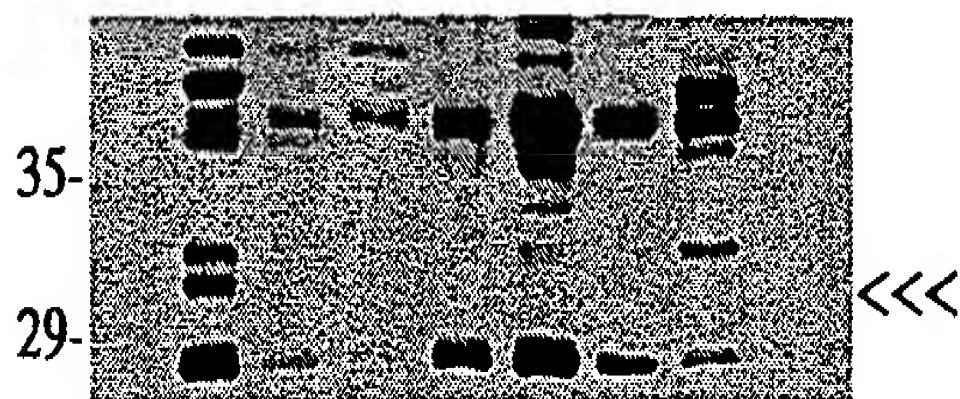
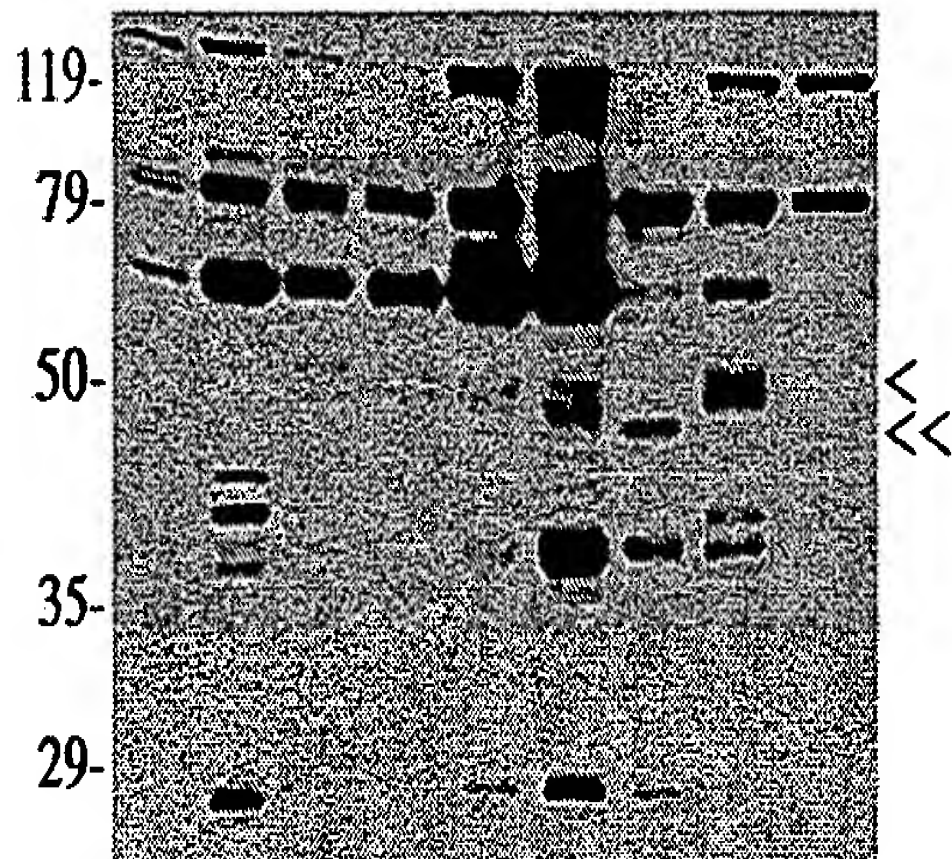




23/24

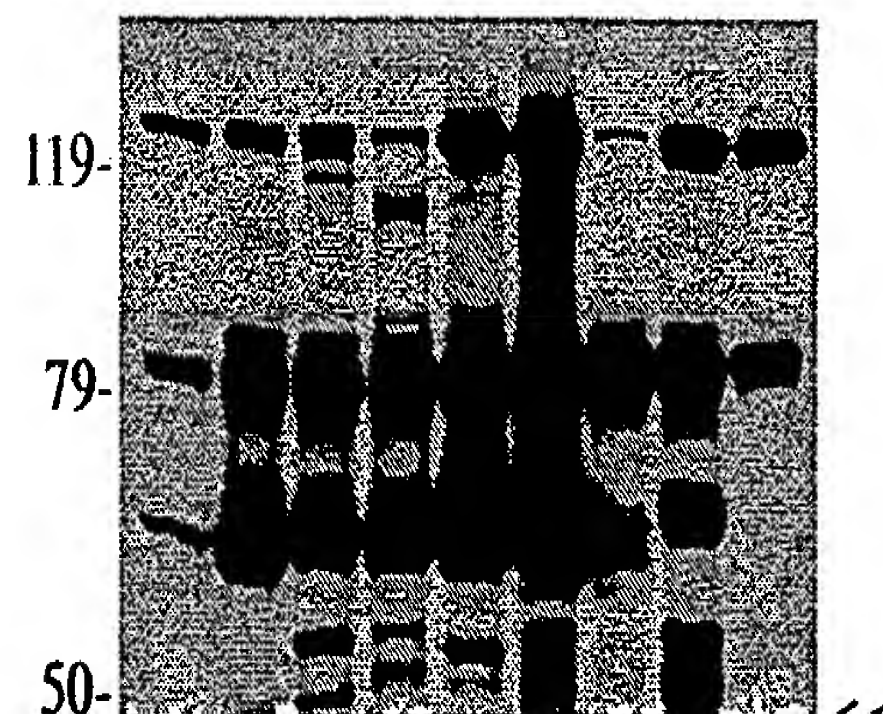
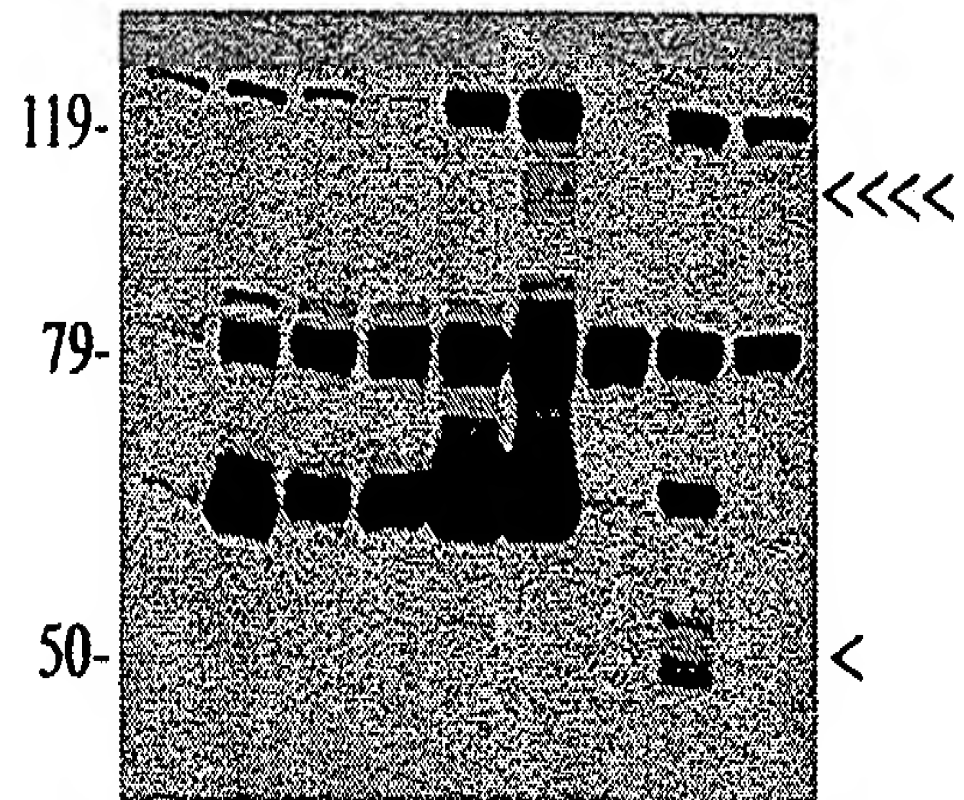
Testis
Testis
Prostate
Lung
Liver
Kidney
Heart
Brain
Brain

+ - - - - + Preheated



Testis
Testis
Prostate
Lung
Liver
Kidney
Heart
Brain
Brain

+ - - - - +





24/24

RECEIVED
APR 17 2003
TECH CENTER 1600/2900

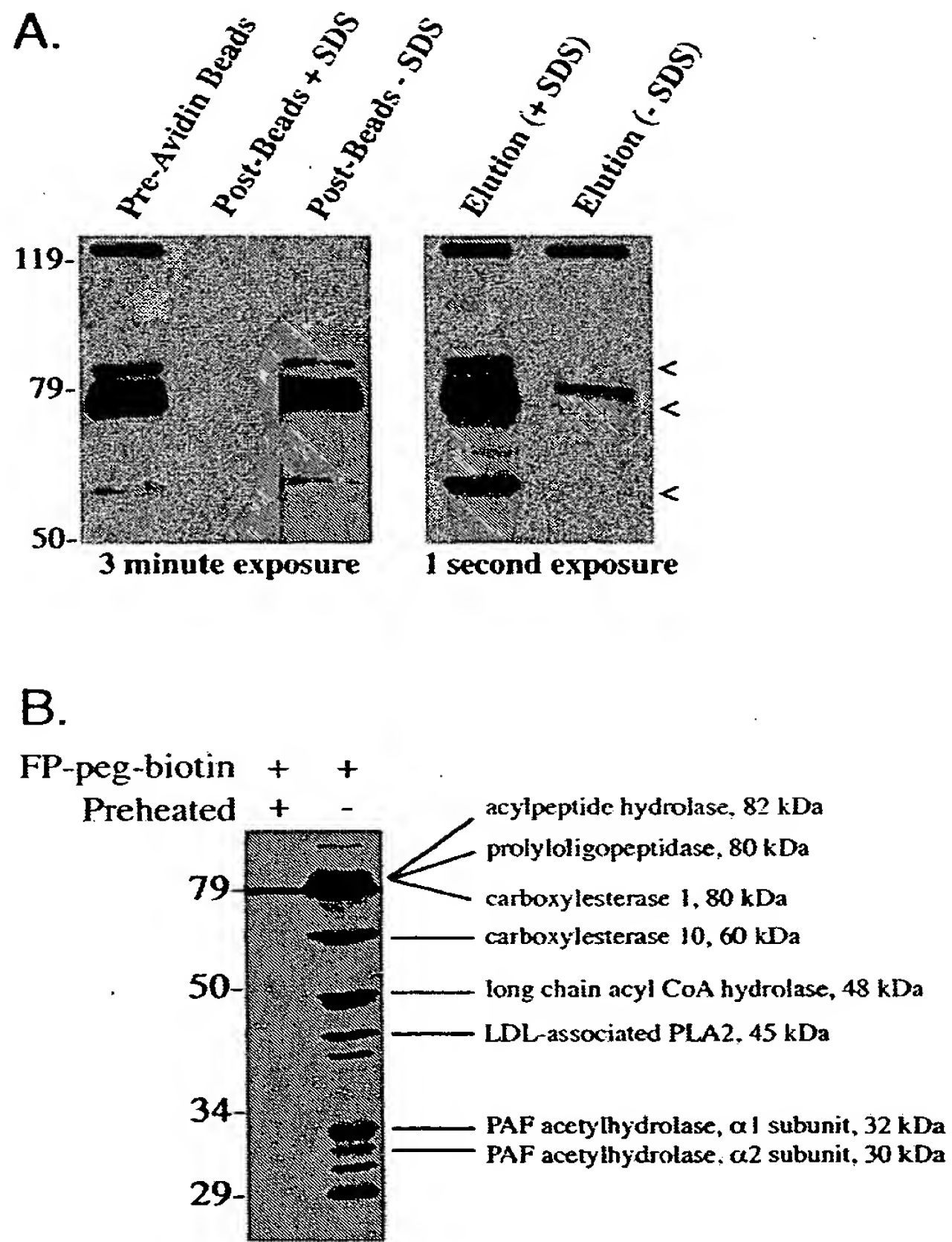


FIGURE 23